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The natural cross-pollination of crop plants with particular reference to the radish

BY M. B. CRANE AND K. MATHER, *John Innes Horticultural Institution, Merton*

(With 6 Text-figures)

Experiments were carried out to determine what degree of intervarietal cross-pollination occurs when two radish varieties are grown at varying distances apart. The varieties chosen were Icicle and Scarlet Globe. Hybrids are distinctive; thus crossing is readily determined.

In closely planted experiments, where one half of the plot was Icicle and the other half Scarlet Globe, the amount of intercrossing ranged from 30 to 40% at a distance of 9 in. to an average of about 1% at 15 ft. Intercrossing decreased fairly regularly and rapidly with distance.

Single plants of Icicle were run out from plots of Scarlet Globe; in 1941 at distances from 9 in. to 95 ft. and in 1942 from 9 in. to 408 ft. In the plants near the red plots crossing was approximately 100%. In 1941 there was a regular and rapid fall with distance and intervarietal crossing stopped at 95 ft. In 1942 the plots were closer to beehives; intercrossing was less regular and extended farther than in 1941, but beyond 240 ft. it was only 0·1%, and in an all-white plot 360 ft. from a red plot with no intervening stringer plants it was nil.

Given a mass of plants and a profusion of flowers, bees confine their flower visiting to a small area. When radishes are grown in quantity 300 ft. guards against contamination of stock due to intervarietal crossing. If only small numbers are grown intercrossing is likely to occur over a greater distance.

Two kinds of practical problems arise in the pollination of crop plants: (1) in crops which are propagated vegetatively, such as fruit trees, where owing to the prevalence of self- and cross-incompatibility it is necessary to plant different varieties of the right kinds together to ensure cross-pollination; (2) in crops raised and grown for seeds, such as Brassicae, carrots, etc., where to maintain purity of stock different kinds and varieties must be grown apart to prevent cross-pollination.

With vegetable seed crops common questions are: What kinds readily cross together? How far must different kinds or varieties be grown apart to prevent the occurrence of cross-pollination? Precise answers are difficult owing to lack of information. Experiments were carried out to determine what degree of natural cross-pollination takes place when different varieties of radish are planted at varying distances apart. The results are given in this report.

MATERIALS

The radish proved to be a convenient plant as it has a number of distinct varieties, seedlings grow rapidly and large numbers can be grown on a small area of land, and with glasshouse space available during the winter months, seedlings can be grown and their root characters recorded at almost any time of the year.

The varieties chosen were Icicle with white, long, tapering roots, and Scarlet Globe, with deep red, short, round roots. These varieties are distinct and intercrossing is clearly indicated by the root characters: crossed seedlings have purple-coloured, intermediate, conical-shaped roots, no matter which variety was the female parent. Scarlet Globe and Icicle breed true to colour, shape and other characters of their roots. They are not pure in the genetic sense, but for horticultural purposes this is not essential and may not be desirable. The flower colours of Icicle are white, lightly tinged or deeply tinged red like those of Scarlet Globe. Bees visit the flowers indiscriminately.

DESIGN OF THE EXPERIMENTS

The experiments were carried out in 2 years, 1941–2. In 1941 they were laid out in four plots as follows:

Plot 1. This plot consisted of 40 rows with 21 plants per row. The first 20 rows were Icicle and the remaining 20 rows Scarlet Globe. Seeds were sown in this and in all other plots in groups of three 9 in. apart, and each group was thinned to one plant about 10 days after germination. The dimensions of the plot were 29 ft. 3 in. long by 15 ft. 9 in. wide, and it contained 840 plants, 420 of each variety.

Plot. 2. A duplicate of plot 1, grown about $\frac{1}{8}$ mile from plot 1.

Plot 3. This plot consisted of 21 rows with 21 plants in each row. The central plant in the middle row and in each outside row was Icicle: the others were Scarlet Globe. The size of the plot was 15 ft. 9 in. by 15 ft. 9 in., and it contained 441 plants, 438 Scarlet Globe and 3 Icicle. Extending from two sides of the plot 14 plants of Icicle were grown, seven plants on the south side and seven on the north side strung out at the following distances from the plot: $\frac{3}{4}$, $2\frac{1}{4}$, $5\frac{1}{4}$, $11\frac{1}{4}$, $23\frac{1}{4}$, $47\frac{1}{4}$ and $95\frac{1}{4}$ ft. This plot was grown about 600 ft. from plot 2.

Plot 4. A duplicate of plot 3, with the exception that only 6 stringer plants run out from the north and south sides. They were grown at the same distances from the plot as in plot 3, the first being $\frac{3}{4}$ and the sixth 47$\frac{1}{4}$ ft. from the plot. This plot was grown 350 ft. from plot 3 and about 1000 ft. from plot 2.

In 1942 the experiments were laid out in three plots as follows:

Plot 5. This plot contained 21 rows with 21 plants in each row; the central plants in the 5th and 17th rows were Icicle, the others were Scarlet Globe. The plot was 15 ft. 9 in. by 15 ft. 9 in. and contained 441 plants, 439 Scarlet Globe and 2 Icicle. Extending at right angles from two sides of the plot 47 plants of Icicle were grown, 24 on the east side and 23 on the south side strung out at the following distances: $\frac{3}{4}$, 2$\frac{1}{4}$, 5$\frac{1}{4}$, 11$\frac{1}{4}$, 23$\frac{1}{4}$, 47$\frac{1}{4}$ ft., and then at 24 ft. intervals up to 479$\frac{1}{4}$ ft. on the south side and 455$\frac{1}{4}$ ft. on the east side.

Plot 6. This plot was all Icicle and contained 196 plants, set in 14 rows with 14 plants in each.

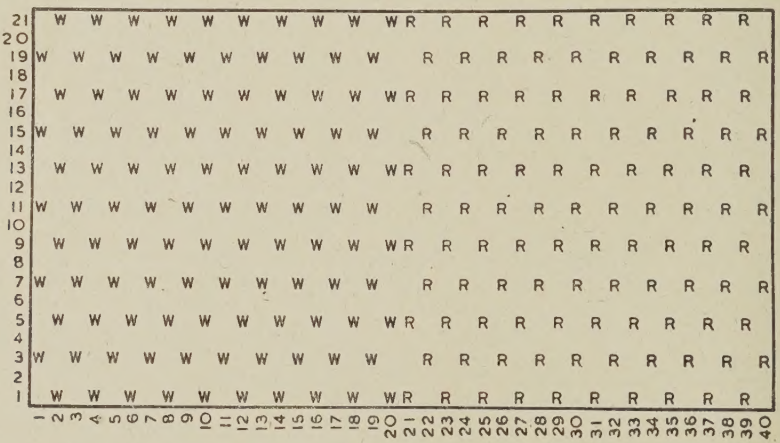
Plot 7. A duplicate of plot 6; 196 plants, all Icicle. The distance between plots 6 and 7 and their distances from plot 5 and from the stringer plants are later given in detail.

Results of the experiments

In plots 1 and 2 the rows were numbered from 1 to 40; the first 20 rows in each plot were var. Icicle and the other 20 rows var. Scarlet Globe. From each plot five equidistant plants were harvested from every odd-numbered row and six from every even-numbered row in the white blocks, while in the red blocks six plants were harvested from every odd-numbered row and five from every even-numbered row. Thus the plants harvested in row 20, the last white row, and those harvested in row 21, the first red row, grew opposite each other; whereas from within the white and red blocks the plants which were harvested grew in alternate positions in adjacent rows. Altogether 440 plants were harvested, 110 white and 110 red from each plot. The position of the harvested plants in each plot is shown in Fig. 1.

From each of the 440 plants which were harvested 40 seeds were sown. The colour and root shape of the resulting plants were recorded and the proportion which had arisen from cross-pollination between the two varieties thereby determined. The results from plot 1 are shown in Fig. 2, and those from plot 2 in Fig. 3.

Experiments with Icicle, Scarlet Globe and their hybrid, had shown the plants to be self-incompatible. No seed is set following self-fertilization. Hence all seed obtained in our experiments was the result of cross-pollination. Where a test seedling was like its mother in root colour it must have arisen from cross-pollination between plants of the same variety. Where a test seedling had a purple root, it must have been the result of cross-pollination between plants of different varieties. Since effective self-pollination does not occur, the frequencies of white and purple progeny, from white mothers, or of red and purple progeny from red mothers will depend on the relative availability of plants of the same and opposite varieties as pollinators. In plots 1 and 2 the marginal red and white rows lie next to one another. Thus a plant in such a row is flanked by others from the same variety, and also has similar plants behind it. In front of it are plants of the opposite kind. Hence we should expect rather more intravarietal crosses than intervarietal ones. Figs. 2 and 3 show that this expectation is borne out: plants in such marginal rows show from 30 to 40% crossing with the other variety. The frequency of intervarietal crossing falls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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Fig. 1. The position of the 110 white and 110 red radish plants harvested from plots 1 and 2, 1941.

off rapidly, at first, as we move farther away from the intervarietal margin in the plots. Some intervarietal crossing occurs even in the outermost rows but is rarely more than 1 or 2%. In other words, plants 15 ft. away from the nearest individuals of the opposite variety show only a small percentage of effect is very small. The distance from the nearest plants of the opposite kind is the main factor determining the frequency of intervarietal crossing.

The proportion of plants resulting from cross-pollination between the two varieties was higher in the white than in the red blocks. Thus in block 1 it

Fig. 2. Distribution of cross-pollination between two varieties of radish, plot 1, 1941.

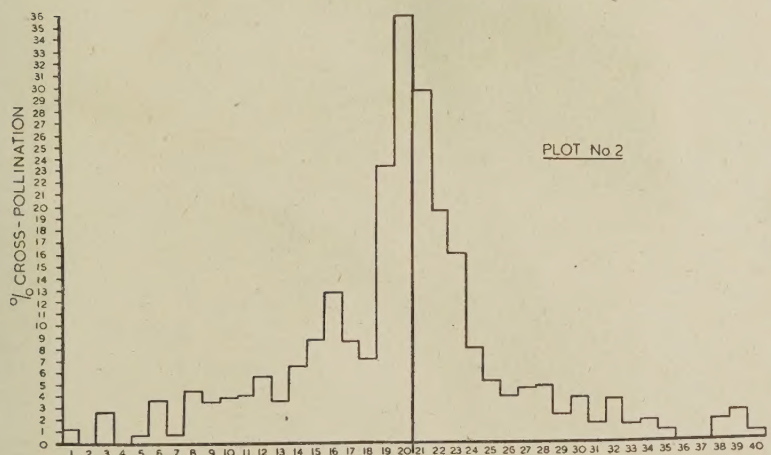

Fig. 3. Distribution of cross-pollination between two varieties of radish, plot 2, 1941.

intervarietal crossing. Most of their pollinations are from the intervening plants of the same kind.

The rate of intervarietal crossing is constant, or nearly so, along the length of each row. There is a little suggestion of higher intervarietal crossing by plants at the end of the row, but, even if real, this was 10·27% from white crossed red, and 7·34% from red crossed white, and in plot 2, 7·07% from white crossed red and 5·63% from red crossed white. In both plots the red variety began to flower 5–6 days before the white variety, and since for this period they could only be pollinated amongst themselves,

i.e. red with red, this might account for some increase in the crossing of white with red. Both varieties flowered profusely and were in flower together for a long time.

Stringer plots. Plot 3 contained 438 plants of other cases. The practice throughout the investigations was to sow the seeds in groups of three, and subsequently to thin out to one plant at each station: in this case the thinning was overlooked and the three plants grew to maturity. Only the white plants,

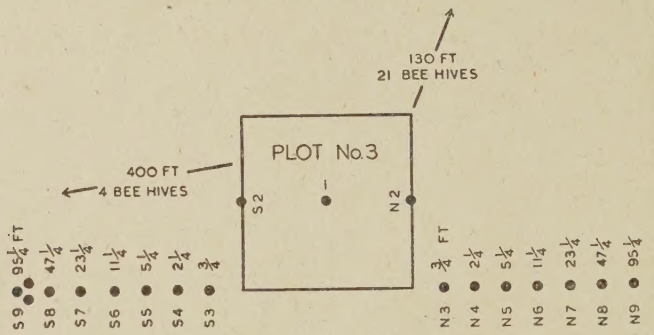

Fig. 4. Lay-out of stringer experiment, plot 3, 1941.

TABLE 1. *Percentage of cross-pollination in stringer experiment, plot* 3, 1941

Plant	Distance from red block (ft.)	White	Purple	% crossed	Plant	Distance from red block (ft.)	White	Purple	% crossed
1	Centre of red block	1	36	97·29	—	—	—	—	—
S_2	In outside row of red block	0	37	100·00	N_2	In outside row of red block	0	35	100·00
S_3	¾	14	24	63·15	N_3	¾	3	29	90·62
S_4	2¼	20	20	50·00	N_4	2¼	12	23	65·71
S_5	5¼	16	20	55·55	N_5	5¼	13	19	59·37
S_6	11¼	20	17	45·94	N_6	11¼	26	11	32·43
S_7	23¾	6	4	40·00	N_7	23¾	32	3	8·57
S_8	47¼	33	5	13·15	N_8	47¼	32	3	8·57
S_9 *A*	95¼	35	2	5·40	N_9	95¼	32	0	0·00
S_9 *B*	95¼	35	0	0·00					
S_9 *C*	95¼	35	0	0·00					

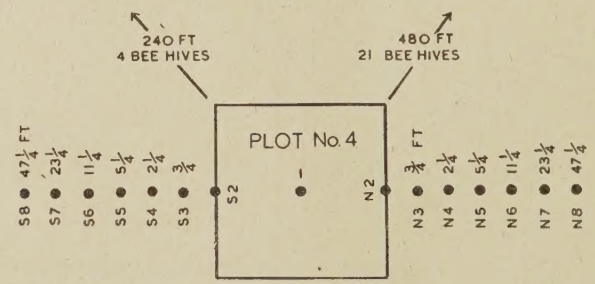

Fig. 5. Lay-out of stringer experiment, plot 4, 1941.

Scarlet Globe and 3 plants of Icicle. The design of this plot, the position of the three white plants in the plot and the distances at which the white stringer plants were run out from the north and south sides of the plot is shown in Fig. 4. In position S_9 three Icicle plants were grown instead of one as in all the stringers and the three in the plot, were harvested; 40 seeds from each of these plants were sown and the results are given in Table 1.

Plot 4, as shown in Fig. 5, was a duplicate of plot 3 with the exception that only six white plants instead of seven were run out from the north and

south sides of the plot. All the white plants were harvested and 40 seeds of each sown: the results are given in Table 2.

In these two plots the frequency of intervarietal crossing of white plants surrounded or nearly so by red ones approaches 100%. This is to be expected as self-pollination results in no seed. Nearly all the available effective pollen is from the opposite variety. The stringer plants can set seed with pollen from other stringers or from the red block. The stringers themselves are progressively wider spaced, as we proceed away from the red block. The first effect of this is to reduce the seed set of the outer stringers: pollen reaches them less often. The exception which proves this rule is afforded by plants S_9 *A*, *B* and *C* in plot 3. These all set well because, thinning being overlooked, each had two close neighbours and effective pollen was plentiful.

The second effect of the wider spacing of the white plants in these experiments was that the rate of intervarietal crossing was much higher on white stringers at 47¼ ft. from the red block than it was on the outermost whites in plots 1 and 2, only 15 ft. from red plants. In the latter case there were plenty of other white plants immediately available as pollinators. In the former case less white pollen was available so that the red blocks exerted an effect over a much greater distance.

TABLE 2. *Percentage of cross-pollination in stringer experiment, plot* 4, 1941

Plant	Distance from red block (ft.)	White	Purple	% crossed	Plant	Distance from red block (ft.)	White	Purple	% crossed
1	Centre of red block	0	38	100·00	—	—	—	—	—
S_2	In outside row of red block	0	37	100·00	N_2	In outside row of red block	No seeds		
S_3	¾	10	29	74·35	N_3	¾	10	25	71·42
S_4	2¼	21	14	40·00	N_4	2¼	20	19	48·71
S_5	5¼	12	25	67·56	N_5	5¼	22	9	29·03
S_6	11¼	24	7	22·58	N_6	11¼	28	11	28·20
S_7	23¼	33	4	10·81	N_7	23¼	28	10	26·31
S_8	47¼	11	25	69·44	N_8	47¼	31	9	25·50

Fig. 6 shows the design of the 1942 experiments. Plot 5 contained 21 rows with 21 plants in each row: 439 plants were Scarlet Globe and 2, the central plants in the 5th and 17th rows, were Icicle. From this plot 23 plants of Icicle were strung out from the south side and 24 plants from the east side at the distances shown in Fig. 6; the last stringer plant on the south side was 455¼ ft., and the last on the east side 479¼ ft. from the plot.

Each alternate plant in alternate rows in plot 5 was harvested and 40 seeds of each were sown. Thus 120 plants were harvested, 4800 seeds sown, and 4381 plants grew and were recorded. Of these 4374 were red—pure Scarlet Globe—and 7 were purple hybrids. The frequency of intervarietal crossing was thus very small, though somewhat larger than that of intervarietal crossing in the two white blocks. This is due to the presence of two white plants within the block and of others closely adjacent to it.

Plot 6 was situated 510 ft. from plot 5. It contained 196 plants, 14 rows with 14 plants in each, and all were the white variety Icicle. Alternate plants in alternate rows in this plot were harvested, and 40 seeds from each were sown. Thus from 49 plants, a total of 1960 seeds were sown; from them 1880 plants were obtained and of these 1778 were white and 2 purple.

Plot 7 was a duplicate of plot 6 and was situated 440 ft. from plot 5. As in the case of plot 6, 49 plants were harvested from plot 7; 1960 seeds were sown, 1690 plants grew and all were white.

As would be expected from the 1941 results the rate of intervarietal crossing is very low when blocks of plants of the same variety are grown 400 and 500 ft. apart.

Four of the stringer plants died before reaching maturity, the remainder and the 2 white plants in plot 5 were harvested; 40 seeds from each were sown. The results are given in Table 3.

As in 1941 white plants situated in or very near red blocks show a high frequency of intervarietal crossing and, even when the stringers up to 100 ft. away are considered, contamination with red pollen is not negligible. In general the frequency of such crossing is very small at distances over 120 ft. Two plants are exceptions to this rule, S_{15} and E_{12}. The former gave 44 purple progeny out of 76 tested—over 50% intervarietal crossing. Its neighbours behaved normally, and it itself was not unusually early in flowering, but it was situated very close to the beehives and in consequence the bees may have tended to treat it preferentially, so providing it with an unusual pollen mixture. The results from E_{12} could not be accounted for in this way. This plant showed 7 intervarietal crosses out of 37 tested seeds. There is no obvious reason for such behaviour, but a single visit by a bee carrying pollen from the red block could give a result of this kind, as it could pollinate many flowers with such pollen.

The two series of stringers, *S* and *E*, differed in one respect. The latter connected the red block to a white block, but the former ran out without any

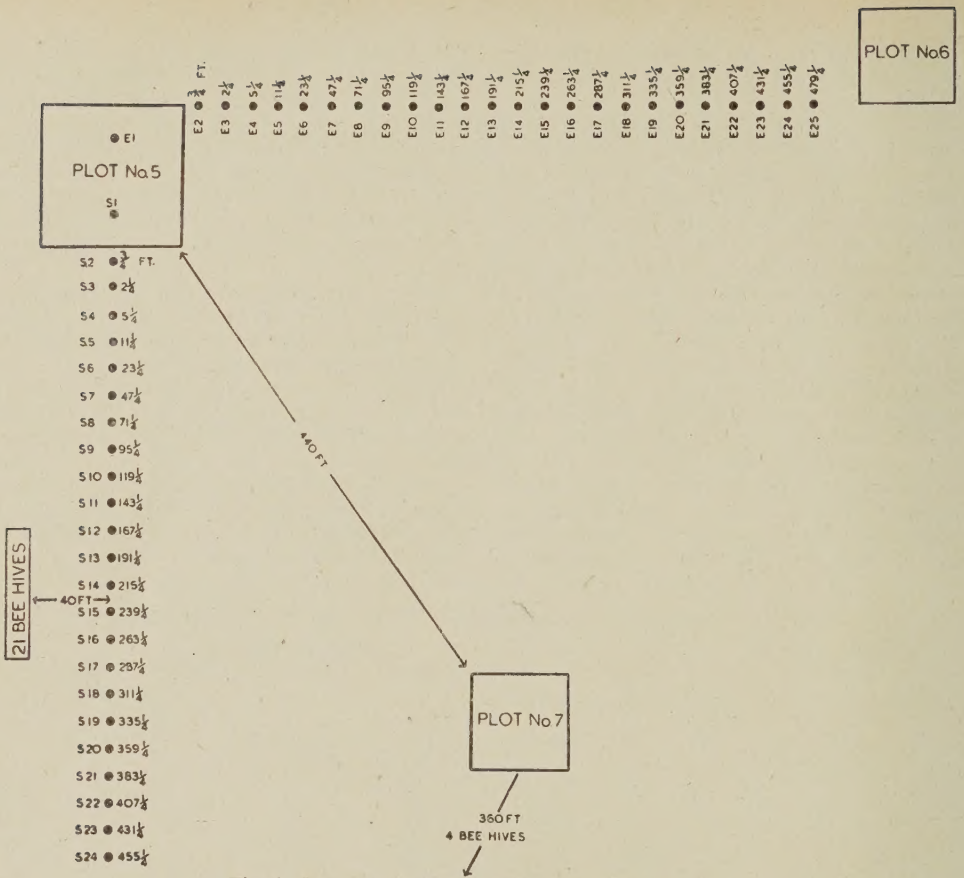

Fig. 6. Lay-out of plot and stringer experiments, 1942.

TABLE 3. *Percentage of cross-pollination in stringer experiments*, 1942

No.	Distance from red block (ft.)	White	Purple	% crossed
E_1	In red block	5	32	86·48
E_2	¾	6	31	83·78
E_3	2¼	10	4	28·57
E_4	5¼	27	10	27·02
E_5	11¼	29	8	21·62
E_6	23¼	27	9	25·00
E_7	47¼	—	—	—
E_8	71¼	34	2	5·55
E_9	95¼	32	2	5·88
E_{10}	119¼	34	1	2·94
E_{11}	143¼	9	0	0·00
E_{12}	167¼	30	7	18·91
E_{13}	191¼	—	—	—
E_{14}	215¼	30	0	0·00
E_{15}	239¼	38	0	0·00
E_{16}	263¼	39	0	0·00
E_{17}	287¼	38	0	0·00
E_{18}	311¼	33	0	0·00
E_{19}	335¼	38	0	0·00
E_{20}	359¼	30	0	0·00
E_{21}	383¼	15	0	0·00
E_{22}	407¼	—	—	—
E_{23}	431¼	—	—	—
E_{24}	455¼	35	0	0·00
E_{25}	479¼	36	1	2·70

No.	Distance from red block (ft.)	White	Purple	% crossed
S_1	In red block	2	15	88·23
S_2	¾	15	25	62·50
S_3	2¼	4	33	89·19
S_4	5¼	23	14	51·85
S_5	11¼	22	10	31·25
S_6	23¼	26	8	23·52
S_7	47¼	24	7	22·59
S_8	71¼	21	17	44·73
S_9	95¼	35	5	12·50
S_{10}	119¼	35	5	12·50
S_{11}	143¼	32	1	3·03
S_{12}	167¼	4	0	0·00
S_{13}	191¼	25	5	16·66
S_{14}	215¼	32	0	0·00
S_{15}	239¼	24	16	40·00
		8	28	77·77
S_{16}	263¼	36	0	0·00
S_{17}	287¼	—	—	—
S_{18}	311¼	40	0	0·00
S_{19}	335¼	36	0	0·00
S_{20}	359¼	31	0	0·00
S_{21}	383¼	37	1	2·63
S_{22}	407¼	38	0	0·00
S_{23}	431¼	—	—	—
S_{24}	455¼	37	0	0·00

such connexion. The effect of this difference is seen in Table 3. The plants E_8, E_9 and E_{10} show a distinctly lower rate of intervarietal outcrossing than do S_8, S_9 and S_{10}. Presumably the bulk of white pollen at the end of the series helped to combat the effect of the red block in the former case. But both series show very low intervarietal crossing at distances of over 250 ft. from the red block. The pollen of the surrounding stringers, at 24 ft. away, is sufficient to overcome the effect of the red block at this distance, though clearly bees do sometimes move over much greater distances as the presence of one purple seedling in the progenies of each of plants E_{25} and S_{21} shows.

Discussion

As the radish is self-incompatible each plant must receive pollen from another plant to produce seed. In these experiments the pollen could come from a sister plant of the same variety or from a plant of a different variety. As shown in Figs. 4–6, 25 hives of bees were maintained close to the experiments. Thus, the bee population was much higher than in most cases where crops are grown commercially for seed, and the number of bees visiting the plots during the flowering period was extremely large. The seed crop was heavy throughout the plots, indicating that cross-pollination had been effectively carried out.

A significant result of these experiments is the regular and rapid fall in amount of intervarietal crossing as the rows of the two varieties were spaced farther apart. In the marginal rows, where the two varieties meet, the proportion of intervarietal crossing ranged from 30 to 40%, whereas in the outer rows, at most 15 ft. away, it was extremely low, averaging about 1% in the outside rows, and in some rows towards the outside being nil. It must be concluded that the area over which an individual bee worked during any one visit was very small. This agrees with the findings of Butler (1943) that bees do not wander over a crop at random, 'but that a foraging bee confines its attention to a relatively small area, usually 4–5 yards in diameter of a crop'. In our experiments the area effectively covered was apparently less than 4–5 yd.

In the 1941 experiments a rapid and fairly regular decrease in intervarietal crossing occurred in the white rooted stringer plants. It reached 100% where the white plants were almost surrounded by red plants in the outside rows of the plots, rapidly falling with distance until at 95 ft. on the north side it was nil.

In the 1942 experiments plants of the white rooted variety were strung out at a greater distance from the plot than in 1941 and they were planted much closer to a strong force of bees. As in 1941 the amount of intervarietal crossing was high in plants near the red variety plot and fell rapidly with distance, but it extended further than in 1941 and the results obtained were more irregular. On the east side there was practically no crossing beyond 170 ft., but on the south side crossing extended further. In particular, plant S_{15} which was 239 ft. from the plot, and of which two lots of 40 seeds were sown, gave a very high proportion of crossed plants. This was due to the very close proximity of this plant to a large number of hives of active bees (Fig. 6). Beyond this plant the amount of crossing was negligible, only one crossed plant being obtained from the seeds sown from 7 plants.

In these experiments, although we had the use of an excessive force of bees extremely close to the experimental plots, intercrossing between the two varieties occurred only within comparatively short distances. Although the experiments were laid out so as to encourage intercrossing, the amount of intercrossing beyond 240 ft. was almost negligible, being only 0·1%, and in plot 6, with which there were no connecting stringer plants, complete purity to type was maintained; no intercrossing occurred between the white plants in this plot and the red plants in plot 5, 360 ft. away. It therefore seems safe to conclude, with seed crops such as the radish, that if varieties are grown in large quantities a distance of 300 ft. between them would be sufficient to guard against contamination of stock due to intercrossing. If only a small number of plants are grown a greater distance would be desirable, since with a few plants contamination due to intercrossing is likely to be more severe, and may even come from a greater distance, than when a crop is grown for seed on a large scale. Thus, a mass of plants and a profusion of flowers, by holding bees to a particular area, plays a considerable part in isolation.

This discussion has been concerned with the problems of the grower of pure seed. His requirements are to have his crops pollinated so that seed is abundantly produced, but at the same time to avoid cross-pollinations with other varieties of the same or allied species. We may now draw some conclusions from the experiments and refer to the pollination problems of the fruit grower which, owing to the clonal reproduction of fruit trees, are in many respects almost the reverse of those of the seed grower.

The radish experiments showed that when there is a profusion of flowers bees confine their foraging and flower-visiting activities to a very small area. It follows, when self-incompatible, or partially self-incompatible varieties of fruit trees are grown, that it is important to plant a sufficient quantity of interpollinating varieties so that ample provision is made for cross-pollination. At best, interpollinating varieties should be planted regularly in equal numbers, but where a smaller quantity of one variety is planted to provide for pollination it is essential that trees of this are systematically and regularly distributed throughout the plantation. Cross-compatible varieties which flower at the same time should be planted

together, and biennial flowering, whereby a variety flowers profusely one year and sparsely the next, should as far as possible be avoided.

We are indebted to Mr A. Gavin Brown for assistance throughout these experiments, to Messrs James Carter and Co. for the seeds of the radishes used in the experiments, and to Mr H. T. Wilkin, a member of the firm, for kindly discussing with us various aspects of the work.

REFERENCE

BUTLER, C. G. (1943). The honeybee and the fruit-grower. *Fruitgrower* **94**, 48–54.

(*Received* 17 *February* 1943)

Studies in the physiology of the onion plant

I. An investigation of factors concerned in the flowering ('bolting') of onions grown from sets and its prevention

Part 2. Effects of day length and temperature on onions grown from sets, and General Discussion

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(With Plate 5 and 3 Text-figures)

This paper completes the survey of the effects of five factors on flowering behaviour, bulbing, and ripening of onions grown from sets (Heath, 1943) and deals with the factors day length and temperature in the second season. The results from both papers are discussed and the main conclusions are as follows: Flowering is greatly reduced or completely inhibited by submitting the plants to high temperature whether (1) during the whole period of growth from seed to set, (2) during the first part or whole of the storage period (Oct.–Mar.), or (3) throughout the second season of growth from set to mature onion bulb. Unless very high temperature (35° C.) is reached, heat storage restricted to the last part of the dormant period may only delay but not reduce flowering. Cold storage (0° C.) throughout or for the first part of the storage period reduces flowering; when restricted to the last part it tends to increase bolting, but at any other period in the life history of the onion plant bolting is apparently favoured by medium temperature (10–15° C.). Long days (16 hr.) throughout the second season inhibit inflorescence emergence irrespective of temperature within the range investigated. Conditions of day length and temperature in the second season favourable to bulbing are inimical to flowering: thus both long days and high temperature tend to encourage bulbing and prevent bolting, while short days inhibit bulbing but allow of bolting if the temperature is not too high. Late planting of sets in the field therefore reduces bolting. Leaf emergence ceases abruptly when bulbing begins at high temperature, and even at low temperature few if any more leaves expand. The relation of this fact to optimal time of planting and the use of American varieties capable of bulbing in short photoperiods is discussed. Seedlings apparently need a longer photoperiod for bulbing than plants grown from sets. A day length just adequate for bulbing at high temperature will not allow of bulbing at low temperature, with the result that seedlings sown very late produce good bulbs at high temperature but may fail to bulb at low temperatures. High-temperature storage, especially during the last part of dormancy, tends to the production of distorted leaves, especially when followed by growth of the sets at high temperature. Such plants show a high mortality. Heat storage also delays and restricts bulbing, which is reflected in the increased number of expanded leaves. Ripening is similarly delayed; this effect is not directly due to water loss during storage.

Heath (1943) described:

(*a*) A preliminary experiment in 1940 upon the effects of high-temperature storage of onion sets.

(*b*) The design of a three-factor and a five-factor experiment (main experiments, 1940–1) undertaken to provide a survey of the effects of temperature and day length upon flowering behaviour, bulbing and ripening.

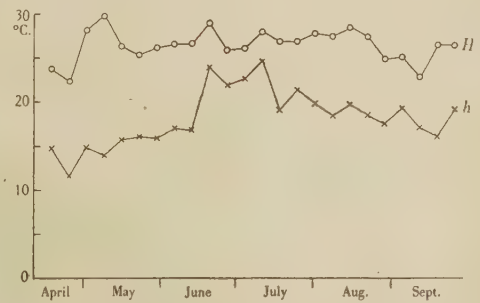
(*c*) The results for the first three of the five factors and their interactions derived from a three-factor field experiment involving (1) time of germination of seed for producing sets, (2) temperature at which sets are grown from seed, and (3) temperature during different parts of the storage period. The present paper describes the greenhouse treatments comprising the remaining two factors (viz. day length and temperature in the second season), and states their results including their interactions with each other and with the previous three factors. The general implications of the results presented in both papers are discussed in relation to those obtained by other workers. Reference should be made to Heath (1943), especially to Table 1, p. 211, where the treatments are summarized together with the symbols by which they are denoted in both papers.

EXPERIMENTAL

Main experiments, 1940–1

Greenhouse treatments in second season. Five-factor experiment

For the greenhouse treatments, sets from all combinations of *I*, *III* and *IV*; *F* and *f*; *C*, t_3, T_3 and T_4 were used (Heath, 1943, Table 1). From each duplicate (A, B) of each of these twenty-four combined treatments four random samples of five sets were taken and allotted to the four day length and temperature treatments, viz. *DH*, *Dh*, *dH* and *dh*. Each such lot of five sets was planted on 16–17 Apr. 1941 in soil contained in a waxed tin measuring $5\frac{1}{2} \times 5\frac{1}{2} \times 8$ in. and fitted with galvanized wire frames for tying up the leaves. The 'high' (*H*) and 'low' (*h*) temperature treatments were located in separate halves of a greenhouse, of which the first was heated by means of water pipes, while the second was unheated and kept as cool as possible by the use of ventilators. In this way a mean temperature difference of about 10° C. was maintained until mid-June, when a spell of hot weather so raised the temperature in *h* that the difference was only 3–5° C. (Text-fig. 1). The tins of plants were placed on the centre bench of the greenhouse from 7 a.m. to 4 p.m. G.M.T. (i.e. 9 hr.) each day and were then placed in a series of eight light-tight ventilated chambers on the north side of the greenhouse. There they received the necessary further hours of artificial light to make up a total of $12\frac{1}{2}$* hr. illumination for the 'short-day' (*d*) treatment and 16 hr. for the 'long-day' (*D*). The source of light in each chamber consisted of two 36 W. gas-filled 12 V. bulbs surmounted by a matt white reflecting surface. The greatest distance of any plant from a bulb was about 3 ft. In the *d* chambers, compensating resistance heaters came on from the time the *d* lights went out until the lights in the *D* chambers also went out, thus ensuring that both *D* and *d* plants were under approximately the same temperature conditions. Furthermore, the two *D* and two *d* chambers in each half of the greenhouse (*H* and *h*) could be interchanged by means of a cross-over switch in the time-switch circuits, and it was therefore possible to rearrange the four lots of tins in each half in order to equalize positional effects as much as possible. In this way the positions were re-randomized weekly. Later in the season, when the late afternoon temperatures in the chambers tended to become too high, the shutters were not put up until considerably after 4 p.m., but the chambers were always closed before the *d* lamps went out (7.30 p.m.). The number of hours of *daylight* received therefore varied somewhat through the season, but was never less than 9 and was always the same for all treatments. Text-fig. 1 shows the weekly mean air temperatures to which the plants were actually subjected (i.e. 9 hr. on the bench and 15 hr. in the chambers each day).



Text-fig. 1. Five-factor experiment, 1941. Greenhouse treatments. Weekly mean air temperatures from thermograph records.

In a number of plants distorted leaves appeared which displayed the following forms:

(1) Individual leaves, especially older ones, were twisted into a flat spiral or cork-screw shape owing to more rapid growth of the abaxial side (Pl. 5, figs. 1–3).

(2) Such individual leaves had a 'waved' appearance such as would result from more rapid growth in alternating sections by the abaxial and adaxial sides.

(3) All the leaves of a plant were twisted spirally round each other to a greater or less extent. This also might be the result of more rapid growth of the abaxial side of the leaf (Pl. 5, fig. 4).

Leaves of types 1 and 2 were easily broken and of

* Owing to a time switch sticking the *d* plants received illumination all night on 19–20 May 1941.

a spongy texture. Many of the distorted leaves of all types were grooved on the adaxial side (Pl. 5, fig. 1).

Counts of plants showing such distortions of the leaves were made on 9 May 1941 and then at approximately fortnightly intervals, two categories being distinguished, viz. 'much-distorted' plants in which at least one leaf was very much distorted; and 'somewhat-distorted' plants with one or more leaves somewhat distorted, or with marked spiral growth of the leaves round each other. Examples of 'much-distorted' plants are shown in Pl. 5, figs. 1–3; a 'somewhat-distorted' plant is shown in Pl. 5, fig. 4.

The percentage incidence of distorted plants at the time of the first counts and also 6½ weeks later are shown in Table 1*a*. The 'much-distorted' plants were at first (9 May 1941) almost confined to the T_4 storage treatment, and they continued to be relatively far more numerous in this than in any other, although later a considerable proportion also appeared among the T_3 sets in *DH* and *dH*. The proportions of 'much-distorted' plants noted increased until 9–10 June, or in some treatments until 23–24 June, when they declined again owing to death of some plants and partial recovery combined with death of the distorted leaves in others. The germination dates (*I*, *III* and *IV*) are not shown separately in the table as they apparently had no consistent effects on the distribution of the distorted plants. While at first there were relatively more 'much-distorted' plants among the *F* sets than among the *f* sets, later the reverse was the case and little importance can be attached to this. The incidence of both 'much-' and 'somewhat-distorted' plants was at first apparently independent of the greenhouse treatments, but later they became much more numerous in the high-temperature (*H*) half of the house. The 'somewhat-distorted' plants increased so much

TABLE 1. *Five-factor experiment*, 1941. *Percentage of plants in greenhouse showing 'much distortion' (heavy type) and any distortion (including both 'much-' and 'somewhat-distorted' plants—ordinary type). Means of germination dates I, III and IV over both duplicates (i.e. means from six tins of five plants each)*

Symbols: *D*, 16 hr. day; *d*, 12½ hr. day. *H*, mean temp. 26° C.; *h*, mean temp. 15° C. *C*, t_3, T_3, T_4, storage temperatures of sets, viz. mean 12·5° C. for 22 weeks; 0–1·5° C. for 22 weeks; 30° C. for 22 weeks; 12·1° C. for 14 weeks followed by 30° C. for 8 weeks. *F*, *f*, mean temperature in first season, viz. 21° C., 14° C.

(*a*) Percentage incidence of distorted plants on particular occasions*

	DH								*dH*							
	C		t_3		T_3		T_4		*C*		t_3		T_3		T_4	
9. v. 41:																
F	—	—	—	—	—	20·0	**26·7**	73·5	**3·3**	3·3	—	—	**3·3**	13·3	**26·7**	63·3
f	—	—	—	—	—	6·7	**6·7**	43·4	—	—	—	—	—	6·7	**6·7**	36·7
Mean	—	—	—	—	—	13·4	**16·7**	58·4	**1·7**	1·7	—	—	**1·7**	10·0	**16·7**	50·0
Mean				**4·2**		17·9						**5·0**		15·8		
23–24. vi. 41:																
F	**4·2**	10·8	—	3·3	**23·3**	83·5	**26·7**	64·2	**5·6**	73·5	—	96·7	**10·0**	100·0	**38·9**	96·7
f	—	6·7	**6·7**	26·7	**17·5**	86·0	**43·4**	89·2	**3·3**	63·5	—	87·0	**24·4**	96·7	**68·7**	100·0
Mean	**2·1**	8·8	**3·3**	15·0	**20·4**	84·8	**35·0**	76·7	**4·5**	68·5	—	91·9	**17·2**	98·4	**53·8**	98·4
Mean				**15·2**		46·4						**18·8**		89·4		

	Dh								*dh*							
	C		t_3		T_3		T_4		*C*		t_3		T_3		T_4	
9. v. 41:																
F	—	—	—	—	—	3·3	**6·7**	33·3	—	3·3	—	—	**6·7**	13·3	**26·7**	46·7
f	—	—	—	—	—	6·7	**6·7**	23·3	—	—	—	—	—	3·3	**10·0**	30·0
Mean	—	—	—	—	—	5·0	**6·7**	28·3	—	1·7	—	—	**3·3**	8·3	**18·4**	38·4
Mean				**1·7**		8·4						**5·4**		12·1		
23–24. vi. 41:																
F	**3·3**	3·3	—	—	—	3·3	**3·3**	45·0	**3·3**	3·3	—	3·3	—	15·0	**6·7**	50·0
f	—	—	—	—	**3·3**	13·3	**10·8**	30·8	—	—	—	—	—	—	**24·2**	47·6
Mean	**1·7**	1·7	—	—	**1·7**	8·3	**7·1**	37·9	**1·7**	1·7	—	1·7	—	7·5	**15·5**	48·8
Mean				**2·6**		12·0						**4·3**		14·9		

* Where one or more plants in a tin had died at the time of the counts the percentage has been calculated on the survivors only.

Table 1 (*continued*)

(*b*) Percentage incidence of distorted plants throughout season, including every plant noted in any count as 'much distorted'*

	DH				*dH*			
	C	t_3	T_3	T_4	*C*	t_3	T_3	T_4
F	3·3	—	33·3	50·0	6·7	—	16·7	66·7
f	3·3	6·7	36·8	66·7	3·3	—	33·3	73·5
Mean	3·3	3·3	35·1	58·4	5·0	—	25·0	70·1
Mean	25·0				25·0			

	Dh				*dh*			
	C	t_3	T_3	T_4	*C*	t_3	T_3	T_4
F	3·3	—	—	13·3	3·3	—	6·7	30·0
f	—	3·3	3·3	16·7	—	—	—	36·7
Mean	1·7	1·7	1·7	15·0	1·7	—	3·3	33·3
Mean	5·0				9·6			

* Percentages calculated on number of sets planted (five per tin).

in *dH* that by 23–24 June almost every plant showed some degree of distortion. The apparent effect of day length on incidence of 'somewhat-distorted' plants indicated by the comparison of *DH* and *dH* (especially in the *C* and t_3 storage treatments) is almost certainly due to its effects on bulbing discussed on pp. 312 and 314–15. The rapid onset of bulbing in *DH*, especially in *C* and t_3, presumably prevented further leaf production, and hence there were no new leaves to become distorted. The data in fact indicate that the distortions are due to high temperature, both during storage (especially when the temperature is high for the last part of the storage period only as in T_4) and during growth.

Table 1*b* shows the percentage incidence of 'much-distorted' plants over the whole season including every such plant noted on any occasion, and in this case the percentages have been calculated on the number of sets planted instead of on those surviving at the time of the counts. The treatment effects are similar to those shown by the data for 23–24 June 1941, but the incidence is considerably higher, partly owing to death or recovery of some of the 'much-distorted' plants between one count and another as mentioned above, and partly no doubt owing to subjective errors in judging the degree of distortion on different occasions. By confining attention to the 'much-distorted' plants however it is certain that all the plants considered were definitely distorted at some period, and it is of interest to examine the proportions of such plants and of normal plants which died during the season or which formed ripe bulbs. Text-fig. 2 illustrates the relative numbers of 'normal'* and 'much-distorted' plants for the four storage treatments under the four combinations of day length and temperature, and also the relative numbers of plants in each category which formed ripe bulbs or which died before the end of the season (30 Sept. 1941). Each histogram includes the three germination times (*I*, *III* and *IV*) and both seedling growth temperatures (*f* and *F*). The relative mortality of the 'much-distorted' plants was far higher than that of the 'normal' plants and also the former produced relatively fewer ripe bulbs.

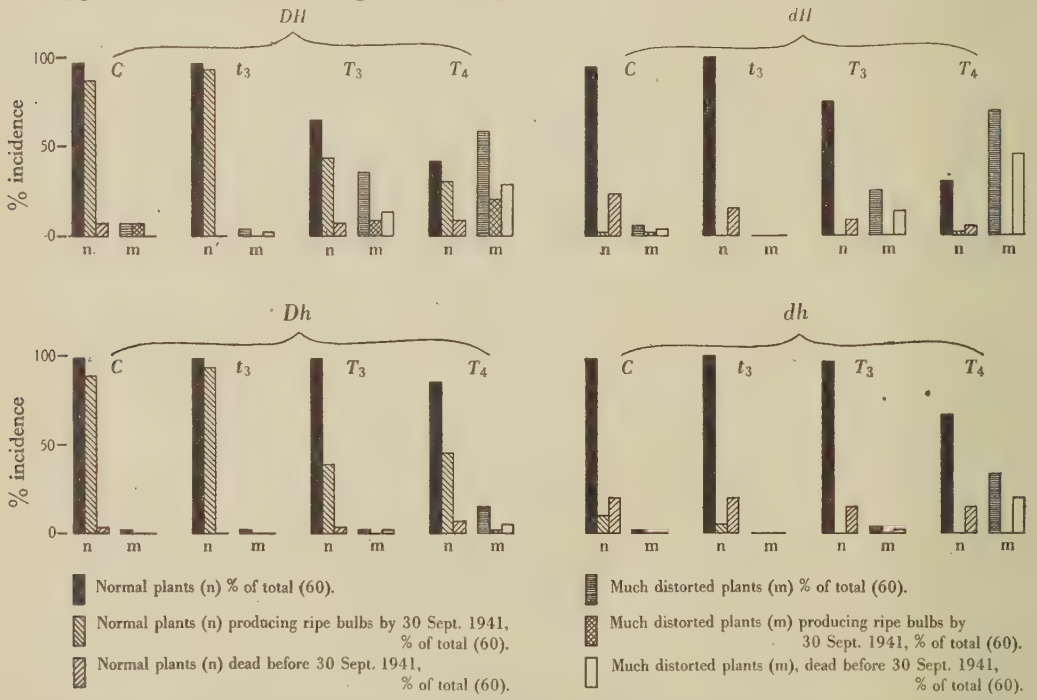
The high mortality among the 'much-distorted' plants suggests that those treatments in which many of them occurred (see Table 1) would have fewer plants surviving to the end of the season. On the whole this was the case, and analysis of variance on the percentage survival data (after angular transformation—see Heath, 1943, p. 217) shows the following significant average effects: day length, *D* (89·4% survival), being above *d* (74·4%); greenhouse temperature, *h* (86·0%), being above *H* (77·7%); and storage temperature, where t_3 (90·8%) is above *C* (85·8%) and T_3 (83·3%), which in turn are significantly above T_4 (67·5%). The interaction between greenhouse temperature and storage temperature also appears to be of importance and consists mainly in the adverse effect of T_4 on percentage survival being aggravated by high greenhouse temperature (*H*) as compared with low (*h*).

Text-fig. 2 also illustrates the important effects of storage temperature and of day length upon production of ripe bulbs, and the treatment effects upon bulbing may now be considered. When an onion set begins to grow it produces a number of leaves whose initials were already present in the bulb. During this process the bulb is depleted and shrivels. After a time, given appropriate external conditions, a new bulb begins to form owing to the swelling of a few leaf bases and scales. Definite swelling of the base

* For this purpose, 'normal' plants include 'somewhat-distorted' plants.

of the plant was first noted in three instances on 19 May 1941, and counts at approximately fortnightly intervals were carried out from 23–24 May onwards. The percentage incidence of plants showing such swelling, calculated on the numbers of plants surviving at the times of the counts, is shown by the time curves marked 'bulbing' in Text-fig. 3. It is apparent that *with the* $12\frac{1}{2}$ *hr. day* (*d*) little bulbing occurred at either temperature, and that with this day length in the cool half of the house (*dh*) there was no bulbing until after the rise of temperature in mid-June (see Text-fig. 1).* When the temperature fell again at the beginning of August, many of these bulbing plants in *dh* lost their swellings. The bulbing bulbing than *C* and t_3, the effect being greater in the case of T_3 than T_4. The incidence of bulbing among these heat-treated sets was lower and later in the cool half of the house (*Dh*) than in the warm half (*DH*), this effect being more marked for T_3 than T_4.

During the normal ripening of the onion bulb the tissues of the neck first soften so that the leaves collapse, die and shrivel, and the bulb enters upon a dormant period. As in the case of bulbs from seedlings (Heath, 1943, p. 213) these changes can proceed in spite of plentiful soil moisture such as was available in the greenhouse experiment under discussion. Fortnightly records were kept of numbers of bulbs with leaves fallen over and with all


DH
dH
Dh
dh
% incidence
100
50
0
C
t_3
T_3
T_4
n
m
Normal plants (n) % of total (60).
Normal plants (n) producing ripe bulbs by 30 Sept. 1941, % of total (60).
Normal plants (n) dead before 30 Sept. 1941, % of total (60).
Much distorted plants (m) % of total (60).
Much distorted plants (m) producing ripe bulbs by 30 Sept. 1941, % of total (60).
Much distorted plants (m), dead before 30 Sept. 1941, % of total (60).

Text-fig. 2. Five-factor experiment, 1941. Greenhouse and storage treatments. Mortality and production of ripe bulbs in normal (*n*) and much distorted (*m*) plants. For further explanation see text.

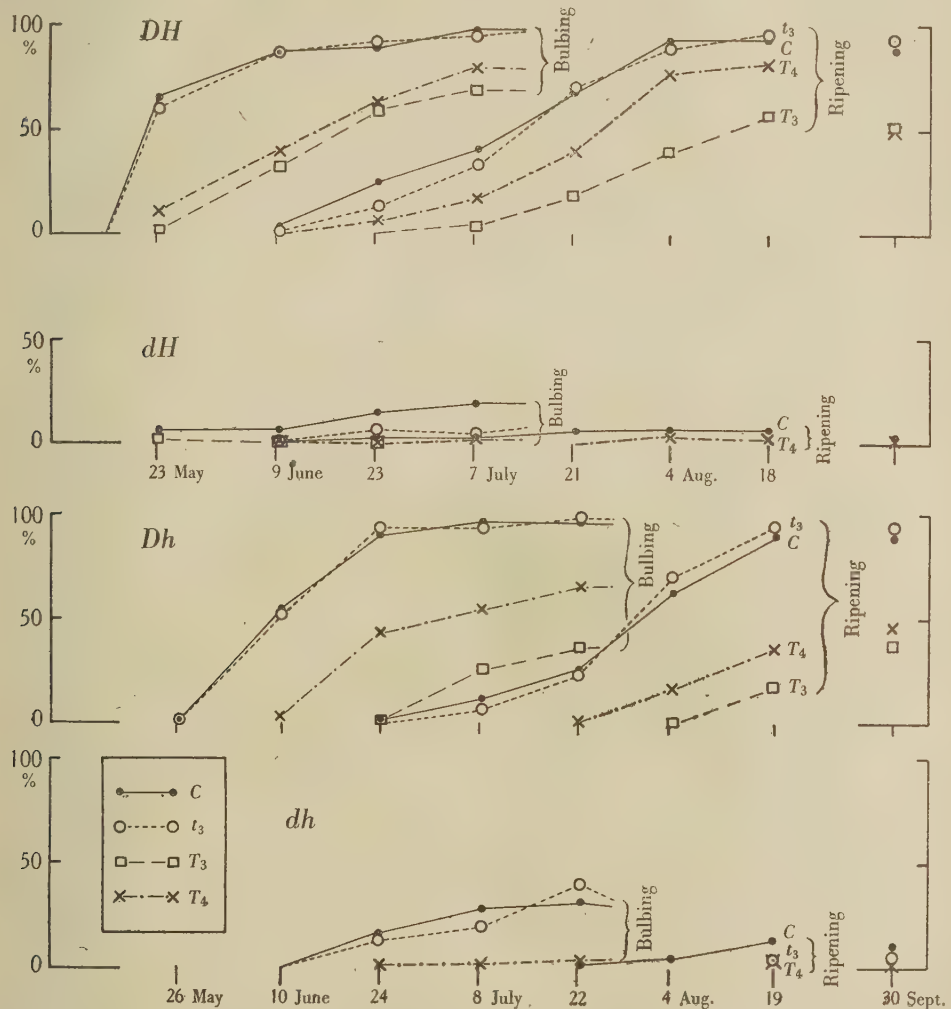
in short days was practically confined to the *C* and t_3 storage treatments. *With the* 16 *hr. day* (*D*) the incidence of bulbing reached nearly 100% among the *C* and t_3 sets in both halves of the house (*H* and *h*), but bulbing began about a fortnight earlier in *H*. This earliness might have been due in part to the more rapid initial growth of the sets in *H* than in *h*. In both *DH* and *Dh* the sets subjected to high-temperature storage treatments showed later and less

* In *dH*, the first bulbing was noted on 23–24 May, only 3–4 days after the time switch sticking (p. 309), and it seems improbable that either this or the bulbing a month later in *dh* was solely due to the single $36\frac{1}{2}$ hr. period of illumination resulting from the mishap.

leaves dead, and the latter were harvested every 2–4 days. They were air-dried and weighed, and at the end of the season were pickled for dissection together with all the other surviving plants. The results of the dissections on this material will be discussed in a subsequent paper.

The percentage incidence of ripe bulbs (i.e. with leaves dead) is shown in Text-fig. 3 by the time curves marked 'ripening' and is calculated on the surviving plants as in the case of the 'bulbing' curves. The final points (30 Sept.) are calculated on the number planted to show the actual production of ripe bulbs per 100 sets planted. The time curves for ripening show substantially similar effects to

those for bulbing. Very few of the bulbs formed in short days (*d*) ripened. The final points (30 Sept.) for the long days (*D*) show the effects of differential mortality. In *DH*, the higher mortality for T_4 than for any other storage treatment has reduced the final percentage of ripe bulbs (calculated on number planted) below the value for T_3. In *Dh*, on the other hand, although the T_4 value is nearer to T_3 than in the curves for percentage calculated on survivors, it is still above it. The best estimates of the potentialities of the treatments for producing ripe bulbs, apart from treatment effects upon mortality, lie between the results given by the two methods of expression. For a given treatment, calculation on the survivors gives an over-estimate, since the 'much-distorted' plants, which were less likely to produce ripe bulbs than the normal plants in the event of survival, had a higher rate of mortality. Calculation on the number actually planted gives an under-estimate, since many of the plants which died would presumably have given ripe bulbs had they survived. From a practical viewpoint the treatment effects upon mortality should be included, and analysis of variance was carried out on the final percentages of ripe bulbs (long days only) calculated upon the number planted (using transformation to angles). Of the main effects two are significant: the effect of temperature in the first season, *f* (73·8 %),



Text-fig. 3. Five-factor experiment, 1941. Greenhouse and storage treatments. Percentage incidence of bulbing and ripening. (Means of germination dates *I*, *II* and *IV*, and seedling growth temperatures *F* and *f*.) For further explanation see text.

being above F (63·8%), with a value of P less than 0·05, and the effect of storage temperature ($P<0·001$). The storage treatment means are shown in Table 2, together with the corresponding mean percentage survival data for comparison, the significant differences among the former being indicated at the foot of the table. The untransformed data are not sufficiently consistent to serve as a basis for discussing interactions.

Apart from the question of the *proportion* of sets giving ripe bulbs, the *time* taken to form and ripen the bulbs is of interest, as was indicated by the field results for premature ripening (Heath, 1943). Analysis of variance was therefore carried out on the mean numbers of days from planting to ripening for those sets which formed ripe bulbs (long days only). Of the interactions, two are significant, viz. the two-factor interaction between temperature in the first season and greenhouse temperature (*Ff* versus *Hh*;

TABLE 2. *Five-factor experiment*, 1941. *Percentage of sets planted in greenhouse which had formed ripe bulbs by* 30 *Sept.* 1941. (*Long days only.*) *Also percentage survival* (*in brackets*)

Symbols: storage temp. C, 12·5° C. mean for 22 weeks; t_3, 0–1·5° C. for 22 weeks; T_3, 30° C. for 22 weeks; T_4, 12·1° C. mean for 14 weeks followed by 30° C. for 8 weeks.

Storage treatment means over all levels of other factors (i.e. from twenty-four tins of five plants each)

C	t_3	T_3	T_4
88·4 (95·0)	93·4 (99·2)	45·0 (87·5)	48·4 (75·8)

Significant differences for incidence of ripe bulbs ($P<0·001$) t_3 and $C>T_4$ and T_3.

$P<0·01$); and that between storage treatment and greenhouse temperature (T–t versus Hh; $P<0·05$). Of the main effects, storage treatment is significant either compared with its interaction with greenhouse temperature ($P<0·05$) or with the random variance ($P<0·001$). Both the *Ff* and *Hh* average effects are significantly greater than the random variation but not than their mutual interaction which, however, has only one degree of freedom thus making the comparison very inaccurate. The *Hh* main effect reaches a level of P less than 0·01 when compared with the T–t versus *Hh* interaction, and it is thus of general interest at least where different levels of temperature during the first season are not concerned. The mean numbers of days are 93 for H as compared with 115 for h, showing that at high temperatures the bulbs ripened on the average 3 weeks sooner. The other relevant treatment means are given in Table 3. The *Ff* versus *Hh* interaction (Table 3*a*) takes the form that the F sets ripen more rapidly than the f sets in H whereas, in h, there is no evidence of any such difference. The interaction between storage treatment and greenhouse temperature (Table 3*b*) is such that the T_4 value is nearly the same as that for T_3 in h but considerably lower in H.

The average effects of storage temperature (Table 3*c*) show the delay of ripening by heat treatment, but since the 'error' is based on the T–t versus *Hh* interaction which has only 3 degrees of freedom, none but large differences can be established as significant.

The question of leaf production will be considered in a later paper, but the treatments, especially of day

TABLE 3. *Five-factor experiment*, 1941. *Ripe bulbs formed in greenhouse—mean number of days from planting to ripening* (*long days only*)

Symbols: H, mean temp. 26° C.; h, mean temp. for first 9 weeks 15° C. C, t_3, T_3, T_4, storage temps. of sets, viz. mean 12·5° C. for 22 weeks, 0–1·5° C. for 22 weeks, 30° C. for 22 weeks, 12·1° C. for 14 weeks followed by 30° C. for 8 weeks. F, f, mean temp. in first season, viz. 21° C., 14° C.

(*a*) Means over all levels of germination date and storage treatment (i.e. from twenty-four tins of five plants each)

	H	h
F	89	115
f	97	116

S.E. of a single mean = 1·50
Significant difference required for P 0·05 = 4·5
Significant difference required for P 0·01 = 6·2

(*b*) Means over all levels of germination date and temperature in first season (i.e. from twelve tins of five plants each)

	C	t_3	T_3	T_4
H	85	88	105	94
h	106	105	127	124

S.E. of a single mean = 2·13
Significant difference required for P 0·05 = 6·4
Significant difference required for P 0·01 = 8·8

(*c*) Storage treatment means over all levels of other factors (i.e. from twenty-four tins of five plants each)

C	t_3	T_3	T_4
95	97	116	109

S.E. of a single mean = 2·72
Significant difference required for P 0·05 = 12·2
Significant difference required for P 0·01 = 22·4

length, had such striking effects on the number of leaves emerging that note of the matter is made here. In the experiment under discussion counts of leaves were begun too late to enable the exact relation between bulbing onset and leaf emergence to be elucidated, but with the onset of bulbing, leaf emergence ceased either immediately or soon.* This

* In a subsequent experiment with var. Bedfordshire Champion it was found that in H, plants grown from sets ceased to produce leaves as soon as bulbing was first noted. In h, although in most plants leaf emergence ceased immediately with bulbing onset, some produced one more leaf and in a few cases several more leaves emerged (see Heath, 1943, p. 213, for bulbing and leaf production in seedlings).

accounts for the lower final mean leaf number in long days (*D*) as compared with that in short days (*d*) where little bulbing occurred. The means over all levels of the other factors were: *D*, 10·1; *d*, 16·7. The large and very significant interaction between day length and storage temperature is shown by the values in Table 4. In long days (*D*) the *C* and t_3 leaf numbers are lower than the T_4 number which, in turn, is lower than that for T_3, whereas in short days (*d*) the differences between storage treatments are negligible. These effects are due partly to the differences in *percentage* of plants bulbing and also in part to the *delayed* bulbing of the heat-treated sets (T_3 and T_4) (see Text-fig. 3). (The ripe bulbs produced from heat-treated sets had higher leaf numbers than the *C* and t_3 ripe bulbs.) A similar explanation accounts for the average effect of greenhouse temperature, the mean numbers being *H*, 12·5; *h*, 14·2.

Table 4. *Five-factor experiment*, 1941. *Mean final numbers of emerged leaves on plants grown in greenhouse*

Symbols: *D*, 16 hr. day; *d*, 12½ hr. day. *C*, t_3, T_3, T_4, storage temp. of sets, viz. 12·5° C. mean for 22 weeks, 0–1·5° C. for 22 weeks, 30° C. for 22 weeks, 12·1° C. mean for 14 weeks followed by 30° C. for 8 weeks.

Means over all levels of germination date and temperature in first and second seasons (i.e. from twenty-four tins of five plants each)

	C	t_3	T_3	T_4
D	8·4	8·3	12·7	10·5
d	16·6	17·0	16·6	16·6

S.E. of a single mean = 0·294
Significant difference required for *P* 0·05 = 0·83
Significant difference required for *P* 0·01 = 1·10

The effects of treatment on flowering in the greenhouse will be considered in a subsequent paper in connexion with the numbers of flower initials present in the sets. Here only emergence will be discussed. Not one of the *F* sets produced an inflorescence, nor did any set from the T_3 or T_4 storage treatments. This is in very good agreement with the field results (Heath, 1943, p. 218) except in the case of fT_4. There was complete absence of flowering in long days (*DH* and *Dh*), while in short days at high temperature (*dH*) only one abortive inflorescence emerged. This was in a *IVfC* plant and died shortly after emerging. In short days and low temperatures (*dh*) six inflorescences emerged about the beginning of June. Three of these subsequently died following the rise of temperature in mid-June (Text-fig. 1). They were distributed as follows: two in *IfC*, two in *IIIfC* and two in Ift_3. As percentages over the three germination times (*I*, *III* and *IV*) these give 13·3% for *C* and 6·7% for t_3, the corresponding field results being 14·8 and 6·8%, which is in much better agreement than could be expected with such small numbers.

General discussion

The results presented in this and the preceding paper (Heath, 1943) are discussed together.

(1) *Control of bolting.* The incidence of flowering in onions grown from sets may be reduced in a number of different ways. The effect of size of set on flowering incidence has been established (Boswell, 1923; Thompson & Smith, 1938), and was confirmed by the results of the preliminary experiment (Heath, 1943). The mechanism of this effect is obscure. It does not appear to be a question of the number of leaves emerged or initiated which is an important factor in determining the flowering behaviour of rye and other cereals (Purvis & Gregory, 1937; von Denffer, 1939). Previous workers do not seem to have investigated the effects of the conditions under which the sets are grown from seed, and one of the striking results obtained in the present experiments is the almost complete immunity from bolting of the sets produced at high temperature (*F*) under glass. These sets were smaller than those grown at relatively low temperature (*f*), and it is not possible with the present data to separate the temperature effect from a size effect. Nevertheless, in view of the profound effects on flowering of temperature during storage and the second season, it seems probable that there is a direct effect of temperature apart from size. The practical indication is that sets should be produced under high-temperature conditions, either by the choice of warm districts or the use of glass. In addition to temperature (*Ff*) the other variable factor in the production of sets for the main experiments was date of germination (*I–IV*), this representing a combination of day-length and temperature effects. The higher incidence of flowering found for the *If* sets as compared with *IVf* may be purely a size effect, although the lower temperatures and day lengths to which the *If* seedlings were subjected in the early part of the season may have contributed. On the whole, the effect of germination date on flowering was not great considering the wide range covered (March–May).

The existence of large varietal differences in respect of bolting was shown by Thompson & Smith (1938) and also by the results of the preliminary experiment (Heath, 1943).

(2) *Control of bolting by storage temperature treatment.* Much attention has been paid by other workers to the effects of storage temperatures upon subsequent flowering behaviour. As far as storage throughout the winter at a single temperature level is concerned, the present investigations confirm their results, showing almost complete inhibition of flowering by storage at high temperature (30° C.) and considerable reduction by storage at low (0° C.) as compared with medium temperature (mean 12° C.). Thompson & Smith (1938) experimented also with different temperatures for the last month of the storage period, and Hungarian growers are alleged

to give heat treatment for the last 6 weeks only. There does not appear to be any previous work on the effects of special storage temperatures for the early part only of the winter. The comparison in the present work of the effects of corresponding periods of high or low temperature at the beginning and end of storage is therefore of particular interest (see Heath, 1943, Table 7). With high-temperature storage treatments (T), the effects of 30° C. and 'common' storage temperatures on total flowering are not additive, early heat treatment reducing flowering much more than late. This, coupled with the agreement of the loss-in-weight data with an additive hypothesis, lends no support to the view that the reduction of total flowering by heat treatment is brought about directly by the drying of the sets. The flowering response to early and late cold-storage treatment (0° C.) shows a similar departure from an additive hypothesis, and here there is a suggestion that low temperature given during the last 8 weeks of storage increases flowering. Should this be a real effect it would indicate that the onion is adapted to climatic conditions in which a cold spell in January–February is frequent. That the survival of sets in the field was also greatest for this treatment points in the same direction. The greater effectiveness of early high- or low-temperature storage treatment in reducing total flowering is of theoretical interest in view of the fact that inflorescence differentiation does not begin much if at all before March. The mode of action of high-temperature treatment in preventing flowering cannot, therefore, be simply a matter of 'destruction of the flower bud' as is sometimes stated. There must presumably be actual prevention of inflorescence initiation, though even so it is surprising that the early treatment should be the more effective. The same considerations apply to the effects of cold-storage treatment. Although the flowering response to high-temperature storage treatment shows non-additive effects in the final counts, in the earlier counts the effects appear to be additive, early and late heat treatment giving similar results. Whereas treatment T_2 (first 8 weeks at 30° C.) reduced but only slightly delayed flowering, treatment T_4 (last 8 weeks at 30° C.) delayed flowering but in the end scarcely reduced it,† and T_3 (30° C. throughout) both delayed flowering and reduced it much more than any other treatment. Similarly, with cold storage, t_2 (first 8 weeks at 0° C.) reduced but did not delay flowering, t_4 (last 8 weeks at 0° C.) neither delayed nor reduced flowering (possibly even increasing it) and t_3 (0° C. throughout) slightly delayed and much reduced it.

(3) *Effects of day length and temperature in the second season upon flowering and bulbing.* These are so interrelated that they must be considered together. Thompson & Smith's (1938) results for bulbing show a considerable interaction between day length and temperature. Thus, no bulbing occurred at *any* temperature under the short days of winter and early spring, but under a 15 hr. day bulbing occurred at 70–80° F., delayed bulbing at 60–70° and none at 50–60°. On the other hand, in all their experiments except one, they found no effect of day length on flowering. At first sight the results for flowering in the present greenhouse experiment appear to be in direct contradiction to those of Thompson & Smith, for here inflorescences emerged in the low-temperature half of the house (*h*) under short days but not under long days. At high temperature (*H*) the only inflorescence to emerge, also in short days, was abortive. There was thus a large interaction between day length and temperature with respect to flowering. The apparent discrepancy is resolved if the results of both the present author and of Thompson & Smith are set out in a single two-way table in order of the temperatures and day lengths used. There are then consistent effects of day length and temperature such that both longer days and higher temperatures favour bulbing but discourage flowering. Table 5 illustrates this, and considering the fact that different varieties are involved the agreement is remarkably good.

TABLE 5. *Incidence of bulbing and flowering under different day lengths and temperatures*

	Temperature		
Author and day length	Mean 59° F. (*h*, 17. iv. 41 to 16. vi. 41) 50–60° F.	Mean 72° F. (*h*, 16. vi. 41 to 4. viii. 41) 60–70° F.	Mean 80° F. (*H*, 17. iv. 41 to 4. viii. 41) 70–80° F.
Thompson & Smith, short day (? hr.)	**	*	—
Heath (*d*), short day (12½ hr.)	**	* ()	()
Thompson & Smith, long day (15 hr.)	**	* ()	() ()
Heath (*D*), long day (16 hr.)	() ()	() ()	() ()

** and * indicate much and little flowering; () () denotes much bulbing and () little or greatly delayed bulbing; —, no bulbing or flowering.

The flowering in the cool half of the house (*h*) in the present experiment has been shown as * after 16 June 1941, because half the flowers died off following the rise of temperature. Conditions which

† The very high temperatures of T_6 (up to 35° C.) reduced total flowering. Late heat treatments in the preliminary experiment (T_m and T_h) also show reduced flowering.

encourage bulbing are inimical to flowering and vice versa, this apparent antagonism between the two processes being seen in both Thompson & Smith's results and the present author's. It should be observed that short days combined with high temperatures discourage both bulbing and flowering. Since both long days and high temperatures discourage flowering it would appear that late planting of sets should reduce bolting, and this was found to be the case in a combined time of planting and variety trial. Unfortunately, late planting results in reduced bulb size (see p. 318).

(4) *Effects of environmental factors, other than on flowering*. These effects must be taken into account in considering the most desirable ways of reducing bolting. The production of sets at relatively low temperatures (*f*), especially with late sowing, may lead to excessive numbers of 'thick-necks' and failure of many plants to form bulbs (as in *IVf* and *Vf*). A partial explanation is probably that the low temperatures tend to delay bulb development and ripening until the days are too short for these processes to continue, but there are indications also of an interaction of day length and temperature with respect to bulbing *onset*, such that at lower temperatures longer days are needed (see p. 318). With earlier sowing the sets produced at low temperatures tend to be too large. The production of sets at high temperatures (*F*) results in relatively small and thin-skinned sets which are free from thick-necks. Such sets not only show greater loss in weight than the thicker skinned and larger *f* sets under *all* the various storage treatments, but also a greater increase in loss with the more prolonged heat treatments. These effects on loss in weight are reflected in higher mortality both during storage and in the field, and loss in weight during storage may be taken as a good indication of keeping quality. Thus the data for survival in the field show for *f* sets a beneficial effect of cold storage throughout or for the last part only of the storage period, but no evidence of harmful high-temperature storage effects. For the *F* sets there is, in addition to a similar cold storage effect, a generally harmful effect of heat treatment increasing with longer exposure to high temperature. As in the case of loss in weight data there is no evidence that the effects of 30° C. and 'common' storage temperature upon survival are other than additive, and the effects of heat treatment upon mortality may be due to drying. Since there is no indication that the reduction of flowering with heat treatment is due to drying (see p. 316), the effects of high-temperature storage coupled with high humidity are being tried. The *F* sets, which are so much more susceptible to damage by heat treatment, apparently stand in no need of such treatment since they show no bolting even without it. Furthermore, although they consistently show higher mortality than the *f* sets under the very adverse conditions encountered in the field experiment, in the greenhouse results there is no indication of any such effect. In the case of the T_3 treatment this difference might have been due to the non-random samples of T_3 sets planted (see Heath, 1943, p. 217), but no such explanation is available for the other storage treatments. Two curious effects of temperature in the first season (*Ff*) have been found in the greenhouse experiment (long days only—*D*). The first is that a greater percentage of the *f* sets planted finally formed ripe bulbs. The second is an interaction with greenhouse temperature (*Hh*) such that whereas at high temperature (*H*) the *F* sets ripened more rapidly than the *f* sets, at lower temperature (*h*) there is no evidence of such a difference. Many of the differences in behaviour for the *f* and *F* sets are common to large and small sets generally. Thus small sets dry out more with heat treatment than large, as was found in the preliminary experiment, and the plants they produce are less robust in withstanding adverse conditions in the early stages of growth. They also tend to give smaller yields, if the larger sets do not bolt (Thompson & Smith, 1938) and the *F* sets in the field gave much smaller bulbs than the *f* sets.

The effects of date of germination (*I–IV*) upon flowering were not large, and the only other effect of importance due to this factor was that the sets of the earlier dates showed a smaller mortality under field conditions, this being probably a size effect.

(5) *Effects of storage temperature, other than on flowering*. Several aspects of these require further consideration. There is a significant departure from an additive law of the effects of cold-storage and 'common'-storage temperatures upon final loss in weight, which may, however, be satisfactorily explained (Heath, 1943, p. 216), but in any case the losses are relatively so small that it seems improbable that the effects upon survival (or flowering) would be due to the degree of drying. The latter effects are also non-additive, late cold storage (t_4) enhancing survival more than early (t_2) at least for *F* sets, and early cold storage (t_2) reducing flowering more than late (t_4). The beneficial effect of continuous cold storage upon survival was shown by the t_3 sets in the greenhouse as well as in the field. In the greenhouse the much greater harmful effect of late (T_4) than continuous (T_3) heat treatment, which is unlike the field results,* was associated with the greater incidence of 'much-distorted' plants. This harmful effect in the greenhouse of the T_4 treatment was enhanced by the higher temperature (*H*), again being coupled with more-distorted plants, and very few such plants were seen in the field where the temperatures were lower than in the cool half of the house (*h*). The effects of heat treatment in delaying bulbing and ripening and in reducing their incidence may be of some practical importance. The results of the greenhouse experiment show that *bulbing* in long

* The very severe loss of FT_3 sets in the field was partly due to the non-random selection of these sets for planting (Heath, 1943, p. 217).

days (*D*) is greatly delayed and reduced in incidence by the continuous heat treatment (T_3) and to a lesser extent by the late heat treatment (T_4), these differences being increased by relatively low temperature (*h*) especially in the case of T_3. These effects on bulbing are reflected in the final numbers of emerged leaves, since with bulbing onset leaf emergence ceases either immediately or soon, and although in the field, records of bulbing onset and leaf number were not made, the T_3 plants there soon stood out conspicuously by reason of their large and leafy appearance. The delay of *ripening* by the heat treatments T_3 and T_4 is shown by the greenhouse results for time of ripening of those sets which formed ripe bulbs in long days. There is, however, a curious interaction with greenhouse temperature, for at high temperature (*H*) T_3 delays ripening much more than T_4, but at low temperature (*h*) there is little difference between them. This contrasts with the results for premature ripening in the field, where the temperature approximated to that of *h*, in which T_4 gave far more ripe bulbs by the end of July than T_3. These field results show that the effects of 30° C. and 'common' storage temperatures on time of ripening are not additive, but that the differences are in the opposite sense to those for flowering. Thus late heat treatment (T_4) greatly reduces premature ripening, but early (T_2) has little or no effect, whereas for reduction of flowering the converse holds. As in the case of flowering therefore, there is no indication that the effects of heat treatment in delaying ripening are due to the drying of the sets. Continuous heat treatment (T_3) reduces both flowering and premature ripening almost to zero. Late heat treatment with temperatures rising to 35° C. (T_6) also causes marked reduction of both flowering and premature ripening. The delay of ripening by the T_3 and T_4 treatments proved a distinct advantage in the season under discussion since much larger bulbs were produced, but in a cold wet season it might be disadvantageous. As in the case of bulbing incidence, the percentage of sets forming ripe bulbs in the greenhouse was much lower for the heat treatments.

(6) *Effects of growth temperature and day length upon the interrelations of bulbing, leaf emergence and ripening.* It has been suggested by the results of the present investigations, and confirmed by subsequent experiments, that at high temperatures (*F* and *H*) leaf emergence ceases immediately with the onset of bulbing. At relatively low temperatures (*f* and *h*), on the other hand, one or occasionally several more leaves *may* emerge after the plants first show visible signs of bulbing. These statements are true both for seedlings and for plants grown from sets. Their practical importance is clear, since, at least at high temperatures, the final size of the bulb must be very largely determined by the number of leaves that have emerged when bulbing begins. Under natural day lengths, therefore, the greater the amount of growth before the days are long enough for bulbing to begin, the larger the bulbs will tend to be, especially if hot weather follows bulbing onset—hence the very large bulbs commonly produced by autumn sowing or spring sowing under glass. Hence also the very small bulbs produced by late planting of sets. The effects of day length and temperature upon ripening augment those upon bulbing onset. Thus Magruder & Allard (1937) found that long days in excess of the minimum for bulbing hastened ripening, and in the present experiments very few of the bulbs formed in 'short' (12½ hr.) days ripened at all. With regard to temperature, the ripe bulbs grown from sets at high temperature (*H*) ripened on the average 3 weeks earlier than those at low temperature (*h*), and the early ripening of the sets grown from seed at high temperature (*F*) was also most marked. These effects account for the premature ripening prevalent among American strains when grown in this country, such strains generally having a relatively short photoperiod for bulbing and hence starting to bulb early and tending to ripen off in the long days and commonly high temperatures of June. Thompson & Smith's (1938) results show an interaction between day length and temperature for the bulbing of plants grown from sets, and there was evidence of a similar interaction in the present experiments as far as plants from sets are concerned. The rather crude method of estimating bulbing of seedlings, however, furnished no evidence of any interaction, but here day length was continually increasing and may have reached a value (16 hr.) at which bulbing could occur either in *F* or *f* before the interaction effect could be detected. Clearly, experiments with constant day lengths are necessary to settle this question, and such experiments now being carried out with seedlings of var. Ebenezer show a similar interaction between day length and temperature. In the Ailsa Craig plants grown from sets some bulbing occurred in the 12½ hr. day, whereas in the seedlings the first bulbing seen, in *IF*, was on 17 May (15⅝ hr.). This suggests that seedlings may need a longer photoperiod for bulbing than plants grown from sets, otherwise some *IF* seedlings might be expected to have bulbed noticeably between 25 Mar. (12½ hr.) and 17 May. Such a difference would account for the fact that onions from sets are generally earlier than those from seed (Lloyd, 1914).

The analysis so far presented is empirical, but the problems involved are obviously complex and the necessary preliminary survey has, it is hoped, been provided by the experiments described in this and the previous paper (Heath, 1943). Some of the conclusions reached may need modification for other varieties of onion and for different types of season, but on the whole the results obtained are in agreement with previous work, and it seems likely that the main outlines will prove of wide application.

The author wishes to thank Prof. F. G. Gregory, F.R.S., for his interest and encouragement through-

Fig. 1

Fig. 2

Fig. 3

Fig. 4

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EXPLANATION OF PLATE 5

(All photographs 29 May 1941)

Fig. 1. A 'much-distorted' plant grown in *long* days and low temperature from an onion set given 8 weeks of high-temperature storage at the end of the winter (mid-Jan.–mid-Mar.). Treatment $IVFT_4DhA$. Note spiral curling of leaves due to more rapid growth on abaxial side, and grooving of the adaxial side.

Fig. 2. Another 'much-distorted' plant, grown in *short* days and low temperature, also from a set given late heat treatment. Treatment $IVfT_4dhB$. The 4th leaf has been marked with indian ink.

Fig. 3. Another similar plant. Treatment IFT_4dhB. Note the healthy plant of the same treatment.

Fig. 4. A 'somewhat-distorted' plant growing in the same tin as shown in fig. 3. Treatment IFT_4dhB. Note the spiral growth of the leaves round one another.

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The nutrition of the radish

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Sand-culture experiments with the radish demonstrated that the optimum concentrations of nitrogen for root development were the highest employed, 32·96 and 65·92 p.p.m. Deficiency of nitrogen led to poor growth and to a characteristic outlining of the cotyledons in red, with red petioles. As with the carrot and turnip, a low concentration of available phosphorus, here 2·05–4·09 p.p.m., gave the best root growth; and a marketable radish was obtained with so little as 0·10 p.p.m., suggesting that the relatively large radish seed contains a considerable quantity of phosphorus. The highest concentration of potassium used, 22·44 p.p.m., yielded the largest roots, and a diminution in this amount led to loss of yield and eventually to serious scorch of the foliage. There is possibly some boron in the radish seed; for though absence of this from an otherwise satisfactory solution resulted in diminished yields, the foliage was normal in appearance, and the radish of eatable size; the radishes apparently lacked ability to swell.

The general methods used and the data sought in this investigation have been discussed (Woodman, 1940*a*). The variety of radish grown was 'Turnip, Red, extra early, Short Top Forcing', bought from Messrs Watkins and Simpson.

The concentrations of important and minor elements in the culture solutions have been given (Woodman, 1943, Table 1); there were, however, no reductions in nitrogen for the phosphorus and potassium and boron experiments. To make refer-

ence easy, the variations in the elements under experiment are abstracted from the Table. *Nitrogen experiment*: solution A, 65·92; B, 32·96; C, 16·48; D, 8·24; E, 4·12; F, 0·41; and G, nil, p.p.m. *Phosphorus experiment*: solution A, 16·38; B, 8·19; C, 6·14; D, 4·09; E, 2·05; F, 1·02; and G, 0·10, p.p.m. *Potassium experiment*: solution A, 22·44; C, 11·22; D, 5·61; E, 2·81; F, 0·28; and G, nil, p.p.m. *Boron experiment*: solution B, nil, p.p.m.; this experiment was included, as usual, in the potassium one, solution B then varying from solution A only by the absence of boron, present in all other solutions used throughout at 0·0681 p.p.m.

At harvest (27 Mar.), treatments A–E yielded good globular roots (edible portion plus associated root fibre, as in horticultural practice); but one or two plants with D and E showed traces of the red outlining of the cotyledons characteristic of nitrogen deficiency. With F, the roots were the normal globular shape, but tiny, and the deficiency symptom was well-marked. With G, the symptom was still further developed, and the roots were non-swollen.

The range of nitrogen concentrations most suitable for the production of root or top was B–A, 32·96–65·92 p.p.m. (see the six yield subtables for Table 1); there was possibly a bias in favour of A. This behaviour was more reminiscent of the turnip with nitrogen (Woodman, 1941) than the carrot (Woodman, 1943), where a large concentration of nitrogen was prejudicial to root development. Great deficiency in nitrogen resulted in large top/root ratios, i.e. it favoured the top relatively to the root. Deficiency also led, in general, to less bound water.

Table 1. *Summaries of results for nitrogen experiment with radishes. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	4·37	3·86	2·46	1·30	0·76	0·28	0·21	1·89	0·215
		A=B>C>D>F=G; C>D=E; E=F=G								
Roots, FW	SSS	7·50	7·23	5·77	2·75	1·53	0·22	0·11	3·59	0·444
		A=B>C>D>F=G; C>D=E>G; E=F								
Whole plants, FW	SSS	11·87	11·09	8·23	4·05	2·28	0·50	0·32	5·48	0·581
		A=B>C>D>E>F=G								
Tops, DW	SSS	0·30	0·27	0·20	0·11	0·07	0·03	0·02	0·14	0·015
		A=B>C>D>E>G; D>E=F; F=G								
Roots, DW	SSS	0·34	0·34	0·28	0·15	0·09	0·02	0·02	0·18	0·019
		A=B>D>E>F=G; A>C; B=C>D								
Whole plants, DW	SSS	0·64	0·61	0·48	0·27	0·15	0·04	0·04	0·32	0·030
		A=B>C>D>E>F=G								
Top/root, FW	SSS	0·62	0·59	0·48	0·75	0·81	3·42	3·05	1·39	0·305
		F=G>A=B=C=D=E								
Top/root, DW	SSS	0·91	0·86	0·75	1·06	1·18	6·94	1·13	1·83	0·893
		F>A=B=C=D=E=G								
Top, % moisture	SSS	93·01	93·11	91·85	91·36	91·10	90·17	91·83	91·78	0·198
		A=B>C=G>E>F; A=B>C=D=G; D=E>F								
Roots, % moisture	SSS	95·38	95·28	94·94	94·04	94·01	93·01	77·32	92·00	0·765
		A=B=C=D=E>G; A>F>G; B=C=D=E=F								
Whole plants, % moisture	SSS	94·54	94·50	94·01	93·11	92·87	91·00	88·22	92·61	0·316
		A=B=C>E>F>G; C=D; A=B>D=E>F								

Nitrogen experiment

There were 112 waxed plant-pots arranged as a randomized block of the 7 treatments replicated 16 times. Seed sown, 6 Feb. 1940; germination by 20 Feb.; seedlings singled to one per pot, 23 Feb.; and cultures harvested, 27 Mar. 1940. Each culture received 5 l. of its appropriate solution in lots of 250 c.c. about 3 times weekly.

By 8 Mar. cultures with treatments A–E were normal in appearance, with foliage of a good green colour; they decreased in size as the nitrogen concentration diminished. F and G gave smaller cultures, with well-defined red or scarlet edges to the cotyledons seemingly characteristic of deficiency of nitrogen with radishes, at any rate for this variety (Woodman, 1940*b*). By 15 Mar. the petioles with F and G were scarlet also, and suffusion of red over the cotyledons was taking place.

Phosphorus experiment

The 70 glazed culture jars were arranged as a randomized block of the 7 treatments replicated 10 times. Seed sown, 28 Jan. 1941; germinated by 8 Feb.; seedlings singled, 11 Feb.; and cultures harvested, 17 Mar. 1941. Each culture received 10·8 l. of its appropriate solution in lots of 400 c.c. about every 2 days.

On 20 Feb. there was little difference in the appearance or size of the cultures. At harvest, 17 Mar.,

tops and roots with A–F appeared to be equal in size. Those with G were smaller, but large for only 0·10 p.p.m. of P; the most feasible explanation is that the large radish seed contains enough phosphorus to produce at any rate a marketable radish (2–3 g.).

The six subtables for yield in Table 2 demonstrated that over the range F–A, 1·02–16·38 p.p.m. of P, there was apparently no response; thus, as five of the comparisons clearly indicated, there was no response above F, 1·02 p.p.m. of P (A=B=C=D=E=F>G). Unless the radish plant needs a remarkably low amount of phosphorus, the seed must contain a considerable amount.

turnip (5·46 p.p.m., Woodman, 1941), but not lettuce (cf. Woodman, 1940*a*, where 43·68 p.p.m. of P was the best for the tops and roots of Cheshunt Early Giant).

Top/root ratios were both uninfluenced (NS) by variations in phosphorus, while moisture contents were lowered by a deficiency such as in G.

Potassium and boron experiments

The 70 glazed culture jars were arranged as 16 replications of a randomized block of the 7 treatments. Seed sown, 19 July 1939; germinated by 26 July; seedlings singled, 29 July; and plants harvested,

Table 2. *Summaries of results for the phosphorus experiment with radishes. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	3·77	4·12	3·68	4·29	4·59	3·75	1·05	3·61	0·362
					A=B=C=D=E=F>G					
Roots, FW	SSS	10·85	10·42	10·17	12·63	12·90	9·76	2·96	9·96	1·153
					A=B=C=D=E=F>G					
Whole plants, FW	SSS	14·62	14·55	13·85	16·92	17·49	13·51	4·01	13·56	1·442
					A=B=C=D=E=F>G					
Tops, DW	SSS	0·27	0·31	0·27	0·30	0·34	0·30	0·10	0·27	0·022
					E>A=C>G; A=B=C=D=F>G; B=D=E=F					
Roots, DW	SSS	0·49	0·50	0·47	0·56	0·56	0·47	0·16	0·46	0·044
					A=B=C=D=E=F>G					
Whole plants, DW	SSS	0·76	0·81	0·74	0·86	0·90	0·77	0·26	0·73	0·061
					A=B=C=D=E=F>G					
Top/root, FW	NS	0·37	0·40	0·38	0·34	0·38	0·40	0·74	0·43	0·113
Top/root, DW	NS	0·60	0·63	0·59	0·56	0·64	0·68	0·98	0·67	0·110
Tops, % moisture	SSS	92·55	92·34	92·40	92·52	92·28	91·80	90·15	92·01	0·373
					A=B=C=D=E>G; F=G; A=B=C=D=E=F					
Roots, % moisture	SSS	95·44	95·13	95·19	95·50	95·59	95·14	93·86	95·12	0·311
					A=B=C=D=E=F>G					
Whole plants, % moisture	SSS	94·63	94·37	94·44	94·76	94·76	94·20	92·95	94·30	0·281
					A=B=C=D=E=F>G					

There was a bias in favour of D and E throughout, and these treatments were isolated for the fresh roots in turn against all others. They were both significant against all other treatments, but there was also very strong significance (SSS) within or between the others; A, which yielded numerically the next largest roots, showed no significance against all others, with significance (SSS) within others. Grouped together, D and E showed very strong significance against all others (A, B, C, F and G), as was to be expected; there was no significance within D and E, as was anticipated by an inspection of the yields, but there was still very strong significance within the others, due no doubt to the low yield, 2·96 g., with G. These isolations, therefore, tended to demonstrate that the optimum range of concentration of phosphorus for the radish is E–D, 2·05–4·09 p.p.m. This is of similar magnitude to the optimum concentrations discovered for the carrot (6·14 p.p.m., Woodman, 1943) and

22 Aug. 1939. Each culture was given 9·5 l. of its appropriate solution in lots of 400 c.c. about 5 times a week.

Cultures with A, C and D, were normal and well-developed throughout the period of growth and at harvest. Treatment E had a tendency to a pale, amber-brown, marginal scorch, but the roots were globular, of good colour, and of eatable size (about 2·5 g.). Plants with F and G showed severe scorch and death of the leaves due to deficiency in potassium, and the roots were tiny and non-swollen. Treatment B, similar in all respects to A except for absence of boron, gave a radish of eatable size, but of poor colour and of a more or less tap-rooted shape with little swelling ability; the tops were normal, but smaller than those with A, C and D. Evidently the relatively large radish seed contains boron.

Table 3 demonstrated that for the tops and whole plants, yields were best with A, C and D for both

fresh and dried matter (A = C = D). Treatment A, however, yielded the largest roots, fresh or dried (A > C = D > B = E > F = G); and, as the root is the important part of the radish, it may definitely be stated that the highest concentration of potassium used was the optimum. Thus diminution of potassium led to a progressive decrease in size of root.

Lack of boron gave a radish equal in size to that with E (B = E), where signs of potassium deficiency were visible on the foliage. As stated above, there is evidently boron in the seed.

Deficiency of potassium led to greater top/root ratios (relatively decreased root growth; F = G > A = B = C = D = E), lack of boron having no effect in this regard. On the other hand, deficiency in potassium caused less bound water in all parts of the plant, lack of boron again having no effect; this was clearly shown by the comparisons for the last three subtables, where A = B = C = D = E > F ⩾ G.

I take this opportunity of expressing my best thanks to my assistants, Mr T. W. G. McKean and Mr J. F. N. Leonard, for their great aid in these investigations.

TABLE 3. *Summaries of results for potassium and boron experiments with radishes. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	4·74	3·63	4·70	5·86	3·50	0·64	0·37	3·35	0·488
		A = C = D > F = G; D > B = E > F = G; A = B = C = E								
Roots, FW	SSS	9·17	2·57	6·38	5·53	2·61	0·10	0·06	3·78	0·707
		A > C = D > B = E > F = G								
Whole plants, FW	SSS	13·91	6·21	11·08	11·39	6·11	0·74	0·44	7·12	0·991
		A = C = D > B = E > F = G								
Tops, DW	SSS	0·37	0·28	0·32	0·40	0·25	0·07	0·05	0·25	0·030
		A = C = D > F = G; D > B = E > F = G; A = B = C; A = D > E; B = C = E								
Roots, DW	SSS	0·40	0·17	0·30	0·28	0·13	0·01	0·01	0·19	0·029
		A > C = D > B = E > F = G								
Whole plants, DW	SSS	0·77	0·45	0·62	0·68	0·39	0·09	0·06	0·44	0·051
		A = C = D > B = E > F = G								
Top/root, FW	SSS	0·56	1·72	0·88	1·41	2·06	7·25	7·39	3·04	0·901
		F = G > A = B = C = D = E								
Top/root, DW	SSS	0·99	1·90	1·16	1·52	2·29	5·59	5·66	2·73	0·741
		F = G > A = B = C = D = E								
Tops, % moisture	SSS	92·21	91·90	93·01	92·97	92·58	83·41	81·02	89·59	2·032
		A = B = C = D = E > F = G								
Roots, % moisture	SSS	95·61	93·00	95·01	94·24	94·28	83·50	76·77	90·34	1·614
		A = B = C = D = E > F > G								
Whole plants, % moisture	SSS	94·42	92·46	94·26	93·86	93·61	84·08	80·92	90·52	2·043
		A = B = C = D = E > F = G								

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Notes on basal rot of narcissus

I. A comparison of various methods of using formalin in connection with the hot-water treatment against eelworm

By Lilian E. Hawker, *Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London, S.W. 7*

Control of basal rot of narcissus following hot-water treatment against eelworm was equally good when 0·5 % formalin was included in the bath or was used as a cold or warm steep immediately afterwards. When the formalin steep was delayed control was less good. All methods of applying formalin in connexion with hot-water treatments used in these experiments were equally harmless to growth and flowering of the bulbs.

Gregory (1932) and Hawker (1935, 1940) showed that basal rot (caused by *Fusarium bulbigenum* Cke. & Mass.) occurring in narcissus bulbs after hot-water treatment against eelworm was reduced by the addition of a fungicide to the bath. Similar results were obtained by European and American investigators (see Moore, 1939). In the author's experiments formalin was used most often since it is at least as effective as certain mercurial compounds and is cheap. In commercial practice the fungicide is usually added to the hot-water bath. Good results were observed at a bulb farm but the method is open to objection when the fungicide is formalin, viz. unpleasant fumes from the warm solution, and loss by evaporation if the bath be used several times without further additions of formalin. The disease was controlled in America (McWhorter & Weiss, 1932; Weiss, 1932) by dipping the bulbs in a cold fungicidal solution after hot-water treatment but it was suggested that it might be desirable to allow an interval of 2 hr. between removal of the bulbs from the hot-water bath and chemical treatment. It was claimed that the net increase in weight during the growing season of bulbs treated in this way was greater than when the fungicide was incorporated in the hot-water bath. This method has the advantage of possible economy of fungicides but involves more labour as the bulbs must be handled twice. Moreover, Wood (1934–5) reported that severe damage sometimes occurred when bulbs were plunged into cold water immediately after hot-water treatment. In three out of four small-scale trials Hawker (1940) obtained slightly less increase in weight during the growing season with bulbs dipped in a cold solution of formalin after the hot-water treatment than when formalin was added to the bath. The amount of flower injury and the number of blind buds were greater with the former treatment in an eelworm infected stock treated early in the season. Doucette (1942) reported variations in tolerance of narcissus bulbs to hot-water treatment with formalin according to the locality in which the bulbs were grown, thus emphasizing the importance of treating bulbs at the time of maximum dormancy (Gould, 1934) which varies with locality. The evidence is conflicting and the writer undertook large-scale experiments during three growing seasons to determine the best method of using formalin in connexion with hot-water treatment. These referred to (1) disease control, (2) effect on increase in weight during the subsequent growing season, (3) quality of blooms and foliage, and date of flowering.

Apart from a few experiments with eelworm-infected or *Fusarium*-infected bulbs, the stocks used were healthy. The experiments were replicated three to five times and the bulbs planted in randomized blocks. Unreplicated experiments with small lots of bulbs were also done.

The following treatments were given:

(i) 3 hr. hot-water treatment at 42–43° C. (H.W.T.);

(ii) 3 hr. hot-water treatment with the addition of 0·5 % commercial formalin to the bath (H.W.T. + F.);

(iii) 3 hr. hot-water treatment followed immediately by 5 min. steeping in a cold solution (0·5 %) of formalin (H.W.T. + C.F.);

(iv) similar to (iii) but with an interval (usually 3 hr. but in two experiments 1 hr. only and in one experiment bulbs were left overnight) between the hot and cold treatments (H.W.T. + delayed C.F.);

(v) 3 hr. hot-water treatment followed immediately by 5 min. steeping in 0·5 % of formalin at the temperature of the hot-water bath, viz. 43° C. (H.W.T. + H.F.).

In a few experiments batches of untreated bulbs were included. The treatments were usually applied during the last two weeks of August which experience has shown to be the period of maximum dormancy when hot-water injury is at a minimum. In one experiment parallel series of bulbs were treated in July, August and September to determine whether, as indicated in an earlier small-scale experiment, 'successive' treatment is more dangerous than

the usual 'simultaneous' treatment if applied before or after the optimum period for the district.

1. Effect of method of treatment on disease control

Experiments with stocks infected with *F. bulbigenum* (Table 1) showed equally good control when formalin was used in the bath or as a cold or warm steep immediately afterwards. Delayed formalin treatment gave less control in four out of five trials and it is possible that the period of 6 hr. between the initial wetting of the bulbs and the application of formalin was sufficient to allow penetration by germ tubes from spores deposited during the hot-water treatment.

number of blind buds was greater in lots undergoing 'successive' treatment than in those given the usual 'simultaneous' treatment when these were carried out at the wrong time.

In eight replicated experiments with healthy stocks of varieties, Victoria, Emperor, Spring Glory, Barrii conspicuus, Barrii Seagull and Leedsii Evangeline, involving about 5000 bulbs, no treatment had a significant effect on either average date of flowering or amount of gain in weight during the following growing season. Small-scale experiments gave similar results. In an experiment with a stock of var. Victoria infected with *F. bulbigenum* there was a significant delay in flowering and reduction of the amount of gain in weight during the growing season in batches given hot-water treatment alone when

Table 1. *Effect of method of application of formalin on number of bulbs rotting after hot-water treatment*

Var.	Victoria	Victoria	Emperor	Spring Glory	Golden Spur	Golden Spur	Seagull
No. per treatment ...	140	80	40	80	50	225	200
Treatment:							
None	2	—	—	—	0	—	—
H.W.T.	85	57	20	10	5	13	10
H.W.T. + F.	2	10	7	3	0	3	2
H.W.T. + C.F.	4	7	11	2	0	6	4
H.W.T. + delayed F.	—	15	18	2	—	10	6
H.W.T. + H.F.	—	—	—	3	—	2	3

2. Effect of method of treatment on growth and flowering

No damage to leaves or flowers was caused by any treatment applied at the correct time, viz. late August. In the single experiment with var. Seagull in which bulbs were also treated at other times, early treatment (July) caused roughening of the leaf surface, splitting of the flowers and accelerated flowering, while there was a number of blind buds in the lots treated late (September). This injury was caused by the hot-water treatment and was independent of the use of formalin. Thus, the evidence from this replicated experiment did not confirm the suggestion of the previous small-scale one that the compared with untreated lots or with batches treated with formalin. This delay was associated with the increased number of diseased bulbs in batches hot-water treated without formalin. There was no significant difference between lots in which formalin was applied in different ways. Thus, formalin may be added directly to the hot-water bath or used as a warm or cold steep immediately after bathing with equally good results, but losses from basal rot may follow if formalin treatment be delayed.

Thanks are due to Mr J. Marshall for carrying out all the routine calculations in connexion with the analysis of variance.

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Notes on basal rot of narcissus

II. Infection of bulbs through dying roots in summer

By Lilian E. Hawker, *Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London, S.W. 7*

Inoculations *in situ* of roots of narcissus plants with cultures of *Fusarium bulbigenum* in early summer gave positive results, as shown by the presence of rotted bulbs at the time of lifting or by subsequent rotting of bulbs in storage. These results were obtained only if the soil was wet at and after the date of inoculation. Cool weather delayed infection. Bulbs planted in the autumn in soil artificially or naturally contaminated with *F. bulbigenum* gave a complete stand in spring but later showed considerable losses from basal rot.

Penetration of narcissus bulbs by *Fusarium bulbigenum* Cke. & Mass. was shown by Gregory (1932) and Hawker (1935) to follow contamination by spores present in the hot-water bath during treatment against eelworm. Hawker (1935) also demonstrated that the fungus could enter via the young roots at a temperature of 20° C. or over. This was confirmed by Fraser (unpublished data) working in this laboratory, who, in experiments in which young roots were inoculated in various ways, obtained 222 diseased bulbs out of 270 inoculated and stored at 25° C., and only 55 out of 270 similarly inoculated and stored at room temperature, with corresponding figures of 12/135 and 3/130 for uninoculated bulbs. She confirmed Hawker's (1935) suggestion, based on Gregory's (1932) results that the tip of the young root is more susceptible than the older parts. Infection through growing roots is unlikely to be important in England, except with forced bulbs, since soil temperatures in autumn are too low. Suitably high soil temperatures are often reached in summer and Hawker (1935) reported one experiment in which six out of twenty-five bulbs rotted in storage after their roots had been inoculated in late May while all but one of the controls remained sound. Var. Evangeline used in that experiment is not particularly susceptible and further work was done with more suitable varieties.

The roots of susceptible varieties (vars. Spring Glory, Victoria and Golden Spur) were exposed in May or June and a sand and cornmeal culture of *F. bulbigenum* (2·5 g. cornmeal, 50 g. sand, 5 c.c. water) was scattered over them, after which the soil was replaced. Roots of control plants were similarly exposed and treated with a sterilized mixture of sand and cornmeal. The bulbs were lifted in July and stored during the summer. Table 1 shows that a high percentage of infection followed inoculation when soil conditions were warm and moist. Some bulbs were rotten when lifted, others were sound but rotted later in storage. The roots of the latter were often rotting when lifted and the rot subse-

Table 1. *Inoculation of roots in early summer*

Var.	Treatment of roots	Date of treatment	Soil conditions	No. treated	No. rotten when lifted	No. rotting in storage
Spring Glory	Inoculated	3. vi. 38	Warm, dry	20	0	0
	None	,,	,,	20	0	0
	Inoculated	7. v. 40	Warm, watered	80	25	33
	None	,,	,,	80	0	1
	Inoculated	15. v. 41	Cool, wet	40	0	11
	None	,,	,,	40	0	0
Golden Spur	Inoculated	7. vi. 38	Warm, dry	20	0	1
	None	,,	,,	20	0	0
	Inoculated	30. vi. 41	,,	20	1	0
	None	,,	,,	20	0	0
Victoria*	Inoculated	17. v. 39	Cool, wet	40	8	27
	None	,,	,,	40	2	16
	Inoculated	5. vi. 39	Warm, dry	30	6	18
	None	,,	,,	30	2	16
	Inoculated	24. vi. 40	Warm, wet	60	21	8
	None	,,	,,	60	10	0

* This stock contained a proportion of naturally infected bulbs.

quently progressed up the roots into the bulbs. If the soil was dry inoculations were seldom successful, while cool weather delayed rotting.

In other experiments (Table 2) healthy stocks of vars. Spring Glory and Emperor were planted in soil from which a stock heavily infected with *F. bulbigenum* had been lifted or which had been artificially contaminated by one or more applications of sand and cornmeal cultures of the fungus. Bulbs were also planted in soil which had produced a healthy stock ('clean soil' of Table 2) or in soil where narcissi had not previously grown ('new soil'). After one or two growing seasons the bulbs were lifted, weighed and stored. In the first spring after planting all the stocks showed a good stand but in those left for a second year blanks occurred among the lots in infected soil. Some bulbs from the infected plots lifted at the end of the first growing period, having shown a complete stand in the spring, were rotten when lifted, or developed the disease in storage. Infection had taken place during the warm weather of early summer and it is probable that the fungus entered via the roots. The roots of sound bulbs from the contaminated plots showed reddish rotten patches which did not always develop further in storage. The difference in percentage gain in weight of bulbs from infected and clean plots is greater than can be accounted for by loss of weight in the rotten bulbs and suggests root injury. Infected roots were found by the end of May in a stock of var. Emperor lifted from artificially infected soil and later some bulbs rotted in storage. The evidence from these experiments strongly supports penetration by the fungus through the old roots in summer, although in the experiments of Table 2 the possibility that the fungus entered through the point of emergence of the roots is not excluded. In the experiments of Table 1 this was less likely and in many instances rotting was seen to progress up the roots into the bulb. Damage to the roots during inoculation could not always be avoided, but in the second series of experiments, roots which were not so treated were rotten when lifted, showing that penetration can take place without artificial root injury.

The fungus is able to survive in the soil between the time of lifting and replanting, since sound bulbs planted in September in soil from which a diseased stock was lifted in July developed the disease in the following summer. A similar delay in attack was described by Hawker (1940) when bulbs contaminated with spores of *F. bulbigenum* and planted in the autumn remained sound until the following summer when some rotted in storage. The period during which the fungus remains active in the soil in the absence of narcissus plants is unknown, but from a comparison with results for other *Fusarium* spp. and for strains of *F. bulbigenum* (Reinking & Manns, 1933, 1934; Reinking, 1934) may be considerable. In the writer's experience infection of a clean stock from the soil only takes place when a diseased stock was previously grown in the same plot or when the plot is artificially contaminated so that, if the fungus is a 'soil inhabitant' (Reinking & Manns, 1933, 1934) under English conditions, it is either not universally present or is present only in small amount.

TABLE 2. *Infection from soil*

Var.	Soil conditions	Years left down	No. planted	Blanks in 2nd growing season	No. rotted when lifted	No. rotted in storage	Increase in wt. %
Spring Glory	Artificially infected	1	100	—	19	20	—
	New soil	1	100	—	3	0	—
	Artificially infected	2	160	26	12	40	13·3
	Naturally infected	2	160	11	3	7	67·0
	Clean soil	2	160	3	3	0	84·0
	New soil	2	160	2	0	0	94·8
Emperor	Artificially infected	2	80	7	0	11	24·6
	Naturally infected	2	80	2	0	0	71·5
	Clean soil	2	80	0	0	0	74·6
	New soil	2	80	0	0	0	102·3

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Factors influencing infection of the tomato by *Verticillium albo-atrum*

By F. M. Roberts, Ph.D., *Rothamsted Experimental Station, Harpenden, Herts*

Infection of tomato plants by *Verticillium albo-atrum* was encouraged by application of nitrogenous manures. Application of phosphate had no significant effect on the progress of the disease, but a deficiency of potash tended to encourage it. Steam-sterilized soil inoculated with *Verticillium* immediately after treatment produced a very high total of infected plants. When inoculation of the steamed soil by *Verticillium* was delayed for 17 days or longer after steaming, the steamed soil was no more favourable for development of the disease than untreated soil. Spread of *Verticillium* from the roots of an infected plant to those of neighbouring healthy plants was hastened by killing the infected plant.

Apart from the work of Bewley (1922) and Ludbrook (1933) on the effect of temperature on wilt disease caused by *Verticillium albo-atrum* there is little precise information on the effect of environmental factors on infection by this parasite (cf. review by Rudolph, 1931).

Soil conditions may influence infection by direct effect on the activity of *Verticillium* in the soil and on its first invasion of the roots, or by indirect effect on the further progress of infection through modification of host nutrition and development. The work described here suggests that the second effect is the more important.

Evidence concerning underground spread suggests that *Verticillium*, like other vascular parasites, has little or no power of growth through the soil, but spreads from one host to another through root contact (Garrett, 1938, 1939). The restriction of *Verticillium* to the vascular cylinder of the host, at least until the last stage of the disease, suggests that the fungus cannot pass from the root of an infected plant to that of a healthy plant in contact with it until the former is moribund. This has been experimentally tested.

Methods

Attempts were made to develop a rapid technique for testing the effect of soil conditions on infection by *Verticillium*. Garrett's tumbler method (1936) proved unsatisfactory for experiments with tomato and antirrhinum seedlings. In the earliest stages of the wilt disease progress of infection in the host tissues is slow and the mycelium of *Verticillium* is difficult to detect. Further, seedlings appear less susceptible to infection by *Verticillium* than older plants. Susceptibility of plants to most root-infecting fungi decreases with increasing age, although Blank (1940) demonstrated that in cotton seedlings susceptibility to infection by *Phymatotrichum omnivorum* increases with age, coinciding with accumulation of carbohydrates in the seedling tissues. He induced a severe attack by the fungus on very young seedlings by adding starch to the soil or the agar substratum in which they were grown. It is probable that infection of tomato seedlings by *Verticillium* may be similarly limited, especially as it was observed during this investigation that increase of susceptibility of older plants to infection by *Verticillium* ensues from the minor advantage of lighting (and consequently of carbohydrate production) some plants have owing to their position on the glasshouse bench.

A technique had therefore to be used whereby older plants were exposed uniformly to inoculum of the fungus under different soil conditions and which permitted them to grow under comparatively natural conditions until disease symptoms were fully developed. Tomato plants (var. Kondine Red) were selected for experiment because of their hardiness and rapid growth under glass and also, with the possible exception of the hop (Keyworth, 1942), because the tomato is commercially the most important plant subject to *Verticillium* wilt in Great Britain.

Seedlings were raised to about the eight-leaf stage in 3½ in. earthenware flower pots filled with glasshouse compost or allotment loam. The experimental containers were wooden 'flats', 14 × 8·5 × 3 in., which were half filled with the various soils to be tested. Over the level surface of the soil in each flat was spread a layer (usually 150 g.) of a culture of *Verticillium* on sand and 3% cornmeal (initial moisture content 65% saturation). The flat was filled with soil from the same stock and was pressed down firmly. On the surface of the soil in each flat were stood six of the small pots each containing a healthy young tomato plant with roots just appearing at the drainage hole in the bottom of the pot. Spaces between the pots were then filled with soil. When the roots of the young plants grew out through the drainage holes they eventually met the layer of fungus inoculum in the flat.

Under conditions favourable for growth of plants and pathogen, i.e. from spring to late autumn, development of disease could often be assessed by external symptoms such as wilting, stunting, and/or dying of the older leaves. Wilting was not an invariable symptom, often being transitory. The yellowing of lower leaves was not a reliable symptom

in experiments involving soil deficient in one or more plant nutrients. In some experiments, especially those carried out late in the year, some plants showing no external disease symptoms were infected. In these experiments brown staining of the wood was a fairly reliable indication of infection by *Verticillium*, though such a stain, especially in the wood of roots and collar, might also be caused through infection by *Colletotrichum atramentarium*. A reliable symptom of severe infection by *Verticillium* in some plants, which does not seem to have been reported previously, is an orange-brown external discoloration of some of the roots.

As macroscopic symptoms proved unreliable, microscopic examination of stems and roots were made for *Verticillium* hyphae in the xylem elements. The roots were sectioned, then the collar, and finally the stem at various heights starting 5–10 cm. above ground level. In recording the results, plants showing only root infections have sometimes been tabulated separately from those showing root and stem infections. Restriction of the parasite to the roots is attributed to host resistance. No cultural test for recovery of *Verticillium* from the vascular cylinder was made, as this had no advantage over direct microscopical examination of tissue sections.

The incubation period necessary for full development of symptoms varied according to the time of the year and was shortest in summer when growth of the plants was most vigorous. Successful experiments were made as late as October–December. Unusually low infection totals were obtained in two experiments when the relatively high mean maximum temperatures of 29·5 and 27·0° C. were experienced during the incubation periods. The highest total infections resulted in four experiments when the mean maximum temperatures were 23·3 (May–June), 23·4 (Apr.–May), 25·2 (Apr.–June) and 25·7° C. (Aug.–Oct.). After inoculation plants were left for four or more weeks before examination. The rate of development of the disease may be gauged from the following observations: tomato plants set in inoculated flats on 8 May showed no hyphae in the vessels after 23 days; after 29 days, before there were any wilt symptoms, hyphae of *Verticillium* were found in some plants in the root and in the stem to a height of 3 cm. above the collar. Wilt developed in a few plants the next day, and shortly afterwards became almost general.

Experimental

Early experiments were preliminary in nature, usually consisting of many different treatments with relatively few plants in each. In addition a number of nutrition experiments were carried out with soil mixed with sand in 1:3 proportion, but this proved so unsatisfactory for normal healthy plant growth that, although useful information on the effect of nutrients was obtained, the method was abandoned.

Exp. 1. *Effect of soil texture and various quantities of inoculum on infection by* Verticillium

Four different quantities of cornmeal-sand inoculum were compared, together with a spore-suspension inoculum, in an organic allotment loam, and in the same diluted with three times its volume of sand. Each series comprised one flat containing six plants (see Table 1).

Table 1. *Effect of soil texture and various quantities of inoculum on infection by* Verticillium

Cornmeal-sand inoculum	No. of plants infected (out of 6): Allotment loam	1 soil: 3 sand
100 g.	5 (1)*	2
150 g.	4	1 (1)
200 g.	4 (1)	1
300 g.	4 (2)	2 (2)
Spore-suspension inoculum	3 (1)	2 (2)
Total infections	20 (5)	8 (5)

* Figures in brackets denote plants infected in the roots only.

Spore suspension was a satisfactory inoculum and the smallest quantity of cornmeal-sand inoculum used in this experiment was sufficient, as no increase in the number of infected plants occurred with increasing amounts. Dilution of the soil with three times its volume of sand decreased strikingly the number of infected plants as well as the severity of infection, e.g. 5/8 plants in the soil/sand mixture were infected only in the roots, and only 5/20 in the allotment soil.

Exp. 7. *Effect of steam sterilization of soil on infection by* Verticillium

Comparison was made between infection in untreated allotment loam, and in the same soil inoculated with *Verticillium* (*a*) immediately, (*b*) 17 days after steam sterilization, and (*c*) 32 days after steam sterilization. Each series comprised three flats of six plants each (see Table 2). Soil inoculated with

Table 2. *Effect of steam sterilization of soil on infection by* Verticillium

Treatment of soil	No. of plants infected (out of 18): Root only	Root and stem	Total
Unsterilized	2	8	10
Sterilized immediately before inoculation	3	13	16
Sterilized 17 days before inoculation	2	7	9
Sterilized 32 days before inoculation	0	5	5

Mean max. temp. during experiment (Aug.–Oct.) 25·7° C.

Verticillium immediately after steam sterilization particularly favours infection. If left for recolonization by other micro-organisms before inoculation with *Verticillium* the steamed soil becomes progressively less favourable for infection by the fungus.

Exp. 8. *Effect of supplementary nitrogen on infection by* Verticillium

The decrease in infection described above from dilution of allotment loam with three times its own volume of sand suggested that nutrient starvation of the plant might render it less susceptible to attack by *Verticillium*. A preliminary experiment on the effect of different organic manures on infection by *Verticillium* showed that those taking up available nitrogen from the soil during the early stages of decomposition tended to depress infection, whereas those releasing available nitrogen tended to increase it. The effect of supplementary nitrogen on resistance of the tomato plant to *Verticillium* was examined. Nitrogen was applied to the allotment soil at the rate of 7·95, 3·97 and 0 g. nitrate of soda (1·31, 0·655 and 0 g. N) per flat. Each treatment comprised three flats of six plants each.

The experiment was carried out from October to December when light and temperature conditions were unfavourable for maximum infection, but the total number of infections was not far below the average. In the maximum, medium, and no nitrogen series the number of infections out of eighteen plants was ten, six and eight respectively. A greater difference between the three groups appeared when the severity of infection was considered: e.g. of ten infected plants in the maximum nitrogen series, seven were severely infected and wilted, and in the medium nitrogen series five of the six infections were severe, whereas in the eight infected plants in the series without nitrogen, only three plants were infected throughout, and five weakly infected, the fungus being confined to the roots. The results indicate that nitrogen starvation tends to confine *Verticillium* to the roots of infected plants.

Exp. 12. *Effect of varying quantities of nitrogen with basal dressing of potassium phosphate on infection by* Verticillium

In this experiment, carried out in allotment loam, nitrogen was added as nitrate of soda at the same rates as in Exp. 8 but with the inclusion of another series receiving nitrogen at one-quarter the maximum rate. The dressings were divided into four parts, the first being applied to the uninoculated seedlings and the last 3 weeks after inoculation. Each series comprised four flats of six plants each. In addition to nitrogen two out of four flats in each series received a basal dressing of 1·07 g. potassium phosphate (see Table 3).

Table 3. *Effect of varying quantities of nitrogen with a basal dressing of* K_2HPO_4 *on infection by* Verticillium

Treatment	No. infected (out of 12)	Total infected (out of 24)
(*a*) Max. N (7·95 g. $NaNO_3$ per flat)	9 (4)*	19 (9)
(*b*) Max. N + 1·07 g. K_2HPO_4 per flat	10 (5)	
(*a*) ½ max. N (3·97 g. $NaNO_3$ per flat)	8 (4)	17 (7)
(*b*) ½ max. N + 1·07 g. K_2HPO_4 per flat	9 (3)	
(*a*) ¼ max. N (1·98 g. $NaNO_3$ per flat)	7 (1)	14 (3)
(*b*) ¼ max. N + 1·07 g. K_2HPO_4 per flat	7 (2)	
(*a*) No nitrogen	7 (1)	11 (2)
(*b*) No nitrogen + 1·07 g. K_2HPO_4 per flat	4 (1)	

Mean max. temp. during experiment (Apr.–June) 25·2° C.

* Figures in brackets denote infected plants which were also wilted.

The relation between nitrogen and susceptibility of plants to *Verticillium* wilt shows clearly. The addition of potassium phosphate did not seem to influence the proportion of infected plants in any series. Of the plants receiving phosphate 30/48 were infected compared with 31/48 of those which received no phosphate.

Exp. 13. *Effect of* NPK *nutrition on* Verticillium *infection*

This was a $3 \times 3 \times 3$ factorial experiment which involved all possible combinations of N, P and K at three different levels, high, medium, and low. Nitrogen was applied (as sodium nitrate) at the same levels as in the preceding experiments, i.e. at rates of 1·31, 0·655 and 0 g. nitrogen per flat. P_2O_5 (as NaH_2PO_4) was applied at rates of 0·655, 0·3275 and 0 g., and K_2O (as K_2SO_4) at rates of 2·62, 1·31 and 0 g. per flat. The experiment was carried out in allotment loam, and replication was at the rate of two flats of six plants each per series, making 54 flats with 324 plants in all (see Table 4).

As before, nitrogen greatly influenced the incidence of infection. This is shown in Table 4 by the numbers, 50, 33 and 18, of infected plants in the 2N, 1N and 0N series respectively. A similar summary of the infection figures for P and K gives 36, 31 and 34 for P, and 31, 31 and 39 for K. Thus phosphate has not influenced the incidence of infection, but the figures for potash suggest that deficiency of this nutrient may have increased susceptibility to infection.

This effect of high nitrogenous manuring may explain the greater prevalence of wilt on soils well supplied with organic matter, as noted by Bewley (1922) in England, and by Brittlebank (1924) in Australia. In Mississippi, Miles & Persons (1932) observed a tendency for *Verticillium* wilt of cotton

to be more severe on the heavier sedimentary and alluvial soils than on lighter sandier types.

Deficiency of available nitrogen was found by Clayton (1923) and Fisher (1935) to inhibit infection of tomato by the vascular parasite, *Fusarium bulbigenum* var. *lycopersici*. Cook (1937) concluded that nitrogen deficiency often failed to inhibit infection, though it checked the development of the disease as expressed by manifestation of symptoms: a conclusion with which the present results to some extent agree. Deficiency of nitrogen seems to limit the eventual number of *visibly* diseased plants not so much by preventing initial infection from the soil as by retarding development of *Verticillium* within the vascular tissues. There is, also, a definite reduction in the *number* of diseased plants, i.e. the effect of nitrogen deficiency is shown by reduction in severity of disease, and by reduction in incidence of disease. Another vascular parasite, *Phytomonas stewarti*, causes most severe wilting in maize plants receiving excess nitrogen; under conditions of nitrogen deficiency, little or no wilting develops (Spencer & McNew, 1938).

TABLE 4. *Effect of* NPK *nutrition on* Verticillium *infection*

Treatment	No. of plants infected out of 12	36	108
2N 2P 2K	7		
2N 2P 1K	5	19	
2N 2P 0K	7		
2N 1P 2K	5		
2N 1P 1K	5	15	50
2N 1P 0K	5		
2N 0P 2K	4		
2N 0P 1K	6	16	
2N 0P 0K	6		
1N 2P 2K	4		
1N 2P 1K	2	11	
1N 2P 0K	5		
1N 1P 2K	1		
1N 1P 1K	2	7	33
1N 1P 0K	4		
1N 0P 2K	6		
1N 0P 1K	4	15	
1N 0P 0K	5		
0N 2P 2K	2		
0N 2P 1K	2	6	
0N 2P 0K	2		
0N 1P 2K	2		
0N 1P 1K	3	9	18
0N 1P 0K	4		
0N 0P 2K	0		
0N 0P 1K	2	3	
0N 0P 0K	1		

Mean max. temp. during experiment (June–Aug.) 27·0° C.

Exp. 11. *Spread of* Verticillium *from infected to healthy plants*

Evidence that vascular wilt fungi spread from plant to plant by root contact and not by mycelial growth through the soil was reviewed by Garrett (1938, 1939). Until death, or approaching death, of the host plant most vascular wilt fungi are confined to the vascular cylinder, and it seems likely that the roots of an infected plant do not become infectious to those of neighbouring healthy plants until the fungus emerges from the vascular cylinder with approaching death of the host. To test this some infected plants were killed by bark-ringing, while others were permitted to survive; the rate of spread of the disease to surrounding healthy plants from *dead* infected plants was then compared with that from *living* infected plants. Tomato plants were grown in glazed crocks of approximately 1⅜ cu. ft. capacity. Into each crock was transplanted one infected plant in the centre with four healthy plants in a ring round it. After becoming established, the central infected plants in half of the crocks were killed by bark-ringing. Fourteen large crocks were set up, and in seven the central infected plants were ringed, so that the total number of originally healthy plants in either half of the experiment was twenty-eight (see Table 5).

TABLE 5. *Spread of* Verticillium *to healthy plants from dead and living infected plants*

Treatment of central inoculated plant	No. of plants infected by spread from central inoculated plant: Root infection only	Root and stem infection	Total no. infected (out of 28)
Not ringed (living)	4	8	12
Ringed (dead)	3	20	23

The figures show that killing the central infected plant hastened spread of *Verticillium* to surrounding healthy individuals. The test was a severe one in that when the first pair of crocks was examined (at the end of June) all the healthy plants had been growing in close proximity to diseased plants for nearly 3 months, and, in half of the crocks, with diseased (dead) plants for 2 months. Pairs of crocks containing ringed and unringed central plants were examined at intervals until October, when the last remaining plants were becoming moribund.

This investigation, which is part of a general enquiry into *Verticillium* wilt, was supported by a grant from the Empire Cotton Growing Corporation, to which grateful acknowledgement is made. I also wish to express my sincere thanks to Mr S. D. Garrett for his helpful criticism and advice throughout the work.

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The appearance and spread of mosaic infection in the tomato crop and the relation to seed transmission of the virus

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(With 5 Text-figures)

Appearance and spread of infection with mosaic-inducing viruses were studied for three seasons in tomato crops under glass. Comparison was made between the reactions of plants raised from virus-free seed and those of plants raised from virus-infected seed, on plots distributed at random in a house in which no precautions against entry and spread of virus were taken. Freedom from mosaic infection was maintained longest in plants raised from virus-free seed. An experiment was carried out after steam sterilization of the soil and under exceptionally favourable weather conditions. Appearance of mosaic symptoms occurred later in the life of the plants in this season and plants raised from virus-free seed did not react differently from other plants.

The location of plants first showing mosaic symptoms was related to the depth and texture of soil beneath those plants.

Tests were made of the apparent virus content of infected tomato seed during germination and differences were found in the persistence of virus during germination in seeds of differing origin.

Apparent, 'delayed' seed transmission of mosaic-inducing viruses occurs in the tomato crop, but as yet, this condition can only be interpreted in terms of differences in the resistance of plants raised from seed of differing origin to the multiplication and systemic spread of those viruses. The use of virus-free seed taken from well-nourished vigorous plants is essential to the production of a virus-free tomato crop under commercial conditions.

INTRODUCTION

The problem of the annual appearance and spread of mosaic infection in the tomato crop still occupies the attention of growers and scientific advisers. The sources of mosaic-inducing viruses in the crop, discussed by Clinch (1941), include virus-infected seed, infected smoking tobacco, infective debris in the soil from previous crops, and host weeds of the virus. Widely divergent opinions exist as to the relative significance of these potential sources of infection and this is particularly true of seed transmission.

It seems generally agreed that the chief vector of these viruses is man, although few data are available concerning the activities of glasshouse workers most likely to be conducive to rapid spread. The extent to which insects spread tobacco and tomato mosaic viruses is uncertain, although this may occur (Bewley, 1922; Hoggan, 1934). Under glass, insects

are almost certainly of minor importance in transmitting these viruses, relative to man's activities.

Seed transmission

On the basis of a few field tests, Westerdijk (1910) concluded that seed transmission of tomato mosaic virus did occur. Allard (1916), Gardner & Kendrick (1922) and Caldwell (1934) failed to confirm this, although thousands of seedlings were raised from seed taken from infected plants, and in some cases were allowed to reach the fruiting stage.

Bewley & Corbett (1930) cited an instance of heavy mosaic infection in seedling plants on a commercial nursery early in the spring, where seed infection appeared to be the only explanation. In one experiment they found that up to 6% of the seedlings raised from infected seed showed symptoms of mosaic infection. Good control of mosaic in field and greenhouse was obtained by Berkeley & Madden (1932) by the use of virus-free seed. Many of their trials with mosaic and streak-infected seed gave negative results, and there was sometimes a delay in symptom appearance, infection not always being apparent until the second truss was in bloom.

Milbrath (1937) recorded mosaic transmission in seed of var. 'Indiana Canner' but in no other variety tested. This is unlikely to be simply a varietal characteristic as Berkeley & Madden (1932), using var. 'Grand Rapids', sometimes obtained positive and sometimes inconclusive results with infected seed.

Symptom appearance delayed until the 4–6 leaf stage was reported by Doolittle & Beecher (1937), who, like Milbrath, found that ageing of the seed gave a lower percentage of infection of the young plants. The former make the reservation that 'the infection in the plants grown from stored seeds cannot at present be definitely attributed to seed transmission of the virus'.

Tests of the seed of virus-infected plants have produced apparently conflicting results. Washings of the seed coat gave negative reactions for virus (Gardner & Kendrick, 1922). Ainsworth (1933) detected virus on the seed coat but not in the embryo, whereas Bewley & Corbett and Berkeley & Madden demonstrated virus in the embryo.

The observations reported below attempt to coordinate some of the results of earlier work.

Methods

Observations were made in a number of glasshouses on the nursery of this Station of the appearance of mosaic symptoms on plants of the tomato crop. The date of appearance of a foliar mottle in the young leaves and the positions in the house of the first plants showing symptoms were recorded. The plants were grown under varying conditions and raised from seed of different origins. With fruiting plants the rigid exclusion of potential sources of virus infection is impracticable when handling large numbers of plants and it is not always possible to interpret fully the data obtained from single experiments of this type. Sufficient data were obtained, however, to indicate some practical measures for the production of tomato crops free from mosaic infection.

Experiment I, 1940

A tomato house 100 × 15 ft. was planted with two varieties of tomato, following commercial practice except that no smoking was permitted, the hands of workers were washed with soap and water before and after handling plants, healthy plants were dealt with before diseased plants, and visitors were excluded from the house. The east border was planted with var. 'J.H.2' ('Potentate × Tuckswood' hybrid) and the west border with var. 'Potentate'. The plants of 'J.H.2' were raised from seed taken by the writer in 1939 from virus-free plants. The latter were vigorous, well-nourished plants and had been tested for virus at the time the fruit was picked, by wiping juice expressed from young leaves on to test plants of *Nicotiana glutinosa* and tomato seedlings. The seed of 'Potentate' was taken in 1939 (not by the writer), and a sample of dry seed ground up with a little distilled water was tested by wiping the paste inoculum on to test plants. Lesions on *N. glutinosa* leaves and a foliar mottle in tomato seedlings indicated that virus was present in the seed of 'Potentate'.

Of each variety, 240 plants were set out early in March, no plant showing evidence of mosaic disease. Periodic inspections were made and on 5 June three plants of var. 'Potentate' showed typical mosaic mottling of the young leaves. All plants of 'J.H.2' appeared clean on this date and the fifth to sixth flower trusses were blooming in both groups of plants. Fig. 1 shows the positions of plants with mosaic symptoms on 5 June, and on 22 June when symptoms had appeared in both varieties.

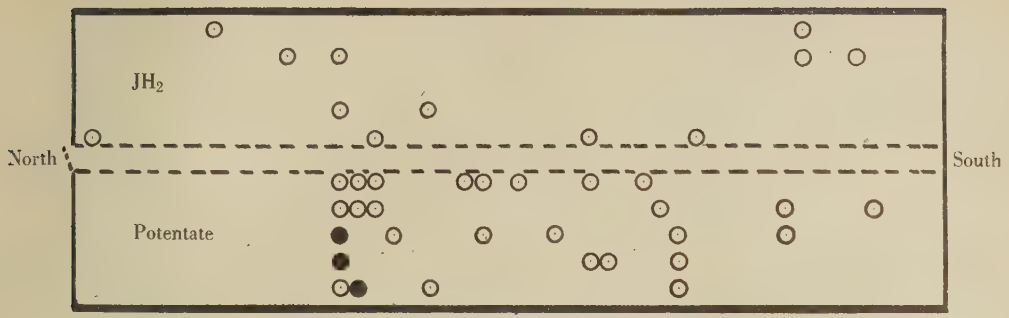
Counts of infected plants were continued until 29 July, when less than 5% of the plants in the house appeared free from disease. The height of the plants was 9–10 ft. when observations were concluded (Table 1).

Table 1. *Number of plants showing symptoms of mosaic infection*

Date ...	June				July				Total no. of plants
	5	22	25	29	9	15	22	29	
'J.H. 2'	0	12	29	49	102	167	222	230	240
'Potentate'	3	28	68	90	126	172	225	233	240

The position of the three plants first showing symptoms of mosaic (Fig. 1) did not bear any visible relation to the position of path, door or hot pipes and, so far as could be ascertained, cultural and manurial treatments were uniform throughout the house. Three potential sources of infection were recognized in this house:

(1) From sources outside the house via insects.



Plants showing symptoms: ● 5 June; ⊙ 22 June.

Fig. 1. Position of plants first showing mosaic symptoms. Tomato house 100 × 15 ft. (not to scale). 5 × 48 plants per border.

(2) From infective debris in the soil from the previous crop.

(3) 'Delayed' transmission of virus by the use of infected seed. Samples of seed from which the first-infected plants were raised were found to contain virus.

Symptom appearance may have been influenced in the first-infected plants by environmental factors peculiar to the west border or certain sections of it, most likely temperature and soil texture. The wall of the west border was also the wall of an adjoining house, whereas the wall of the east border was 3½ ft. distant from the next house. The soil and subsoil of this nursery vary in depth and consistency at different sites on the nursery.

From the data it was not possible to ascertain with certainty the source of infection in this house, and similar difficulties are often encountered on attempting to trace the origin of outbreaks of mosaic infection on commercial nurseries.

EXPERIMENT II, 1941

Observations were made in a tomato house 100 × 27 ft. (House 1, E.R.S.) containing variety trials. More than 1000 plants comprising twenty-nine varieties were grown, and included six varieties (248 plants) raised from seed taken by the writer in 1940 from leaf-tested, virus-free plants. From the original seed stocks of nine other varieties, samples of ungerminated seed were tested for virus and seed of seven varieties gave a positive reaction. Seed samples from the remaining fifteen varieties were not available for testing. The distribution of varieties within the house was random.

The plants were inspected periodically from the time of germination until they were 8–9 ft. high. Symptoms of mosaic infection did not appear until 2 May when the third flower truss was coming into bloom.

The relative position of the varieties in the two borders of the house is shown in Fig. 2, with the

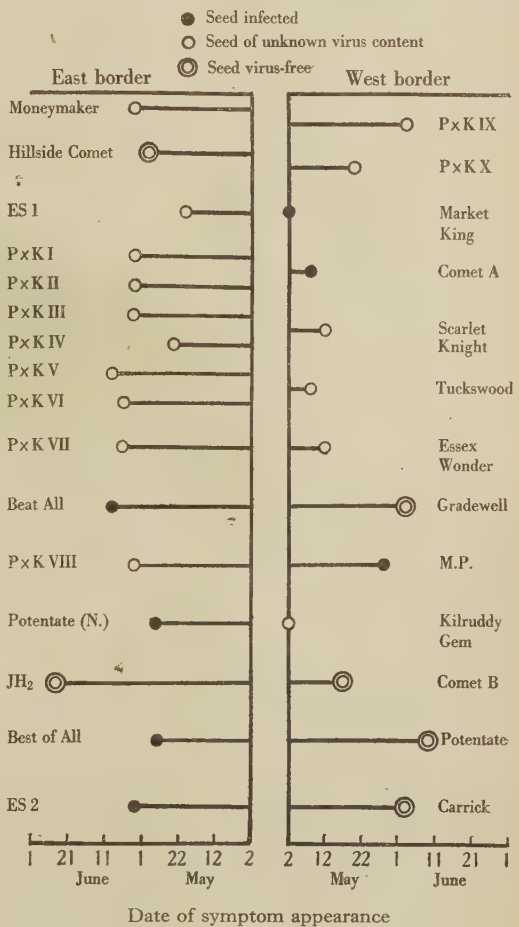


Fig. 2. Relative position of varieties and dates of symptom appearance within varieties.

dates on which symptoms were first noted on any plants within that variety. The position of the plants first showing symptoms is indicated in Fig. 3 for four successive occasions. The counts of diseased plants raised from clean and from infected seed are shown in Table 2 and the percentages of infected plants throughout the season in Fig. 4.

(2) The soil was not sterilized in the winter of 1940–1.

(3) During 1938–41 manurial treatments were uniform.

(4) The grower smoked cigarettes freely when handling plants.

(5) No notice was taken of infected plants by the

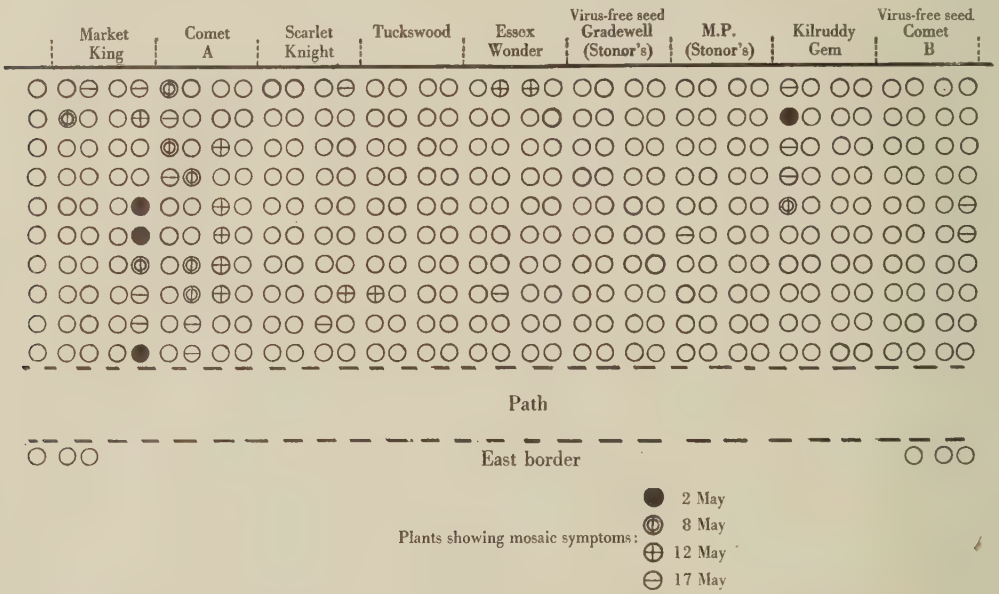


Fig. 3. Position of plants showing symptoms of mosaic infection up to 17 May. (Plants not included in diagram showed no symptoms.)

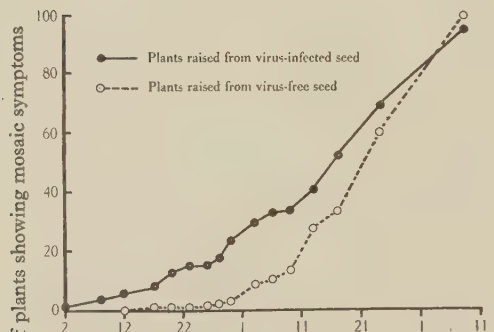


Fig. 4. Appearance of mosaic symptoms in plants raised from infected and virus-free seed.

In assessing the significance of these results the following conditions should be noted:

(1) The house had been laid down to variety trials since 1938, mosaic infection recurring annually with varying degrees of severity.

grower, who worked straight through the house in all cultural operations.

(6) Visitors entered the house freely to inspect the crop.

(7) No attempt was made to control insects.

Thus, conditions were ideal for the entry and spread of mosaic infection from a number of sources.

Figs. 2 and 3 show that plants raised from virus-free seed tended to remain free from symptoms of mosaic infection for a longer period than adjoining plants raised from other seed, despite the complete lack of precautions to prevent the spread of virus through the house. This effect was shown most clearly by vars. 'J.H.2' (Fig. 2) and 'Gradewell' (Figs. 2, 3). Fig. 4 indicates a lower rate of spread within the clean seed varieties during the first part of the infection period, but this result may be partly vitiated by the chance distribution of varieties relative to a soil factor discussed below.

In Exps. I and II there is little evidence that the plants first showing symptoms of infection are the infection foci for all other plants in the house, so that under commercial conditions the roguing of plants showing symptoms is unlikely to prove an effective

means of control. This has been proved true in other houses on this nursery.

Table 2 shows that marked differences occurred between varieties raised from infected seed, both in the dates of initial appearance of symptoms and in the apparent rates of spread within those varieties. Differences also occurred to a lesser extent within the varieties raised from clean seed. The nature of certain factors that may have contributed to such differences was suggested by work described below.

TABLE 2. *Exp. II*, 1941. *Number of plants showing mosaic symptoms*

Var.	May 2	8	12	17	20	23	26	28	30	June 3	6	9	13	17	24	July 8	Total
A. Plants raised from infected seed																	
Market King	3	5	6	10	15	18	18	20	26	33	34	34	37	38	38	38	38
Comet A	0	5	10	13	21	24	24	30	37	39	39	39	40	40	40	40	40
M.P.	0	0	0	0	0	0	0	1	2	6	8	10	19	30	35	40	40
Best of All	0	0	0	0	0	0	0	0	1	2	7	7	10	15	21	34	40
Potentate (N)	0	0	0	0	0	0	0	0	1	2	2	2	3	5	16	37	40
E.S. 2	0	0	0	0	0	0	0	0	0	3	4	4	6	19	35	46	49
Beat All	0	0	0	0	0	0	0	0	0	0	0	2	2	3	13	34	40
Total	3	10	16	23	36	42	42	51	67	85	94	98	117	150	198	269	287
B. Plants raised from virus-free seed																	
Comet B	0	0	0	2	2	2	4	5	5	8	12	14	27	30	38	39	39
Hillside Comet	0	0	0	0	0	0	0	0	2	3	3	4	8	13	28	39	39
Gradewell	0	0	0	0	0	0	0	0	0	6	6	7	19	19	36	40	40
Carrick	0	0	0	0	0	0	0	0	0	4	4	6	11	13	22	50	50
Potentate	0	0	0	0	0	0	0	0	0	0	0	2	4	8	15	40	40
J.H. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	38	40
Total	0	0	0	2	2	2	4	5	7	21	25	33	69	83	149	246	248

Seed transmission and the constitution of the seed

If the 'delayed' seed-transmission hypothesis, suggested by earlier work, is valid, then such transmission only obtains under some hitherto undefined condition of environment, or of the seed, or of both. That differences in the constitution of infected seed occur was suggested by a comparison between the apparent virus content during germination of two batches of seed of different origin.

Seed of var. 'Comet A', from the stock used in Exp. II, was compared with seed of 'Potentate' from plants inoculated with tomato mosaic virus when the first truss was blooming (Selman, 1941). Both groups of seed were taken in 1940 although the parental history of the 'Comet' seed is unknown.

Batches of fifty seeds were germinated in darkness on wet filter paper in Petri dishes. Fifty-seed samples were tested daily for virus content by grinding with a little distilled water and wiping the paste inoculum on to test plants. Two experiments were carried out in July 1941 with similar results. Table 3 presents the results of one experiment together with determinations of seed weights and nitrogen contents (kindly determined by Dr Owen). Tests made on seed coats and embryos separately on the 10th day, showed virus to be present on the coats in both varieties. In no case was virus detected in the embryo of the 'Potentate' seed but in one experiment a positive reaction was noted with the embryos of the 'Comet' seed.

TABLE 3. *Effect of germination on apparent virus content of tomato seed*

(Each sample contained fifty seeds)

No. of days in moist dish	Infection of tomato seedlings: Comet	Potentate	Local lesions on two *N. glutinosa*: Comet	Potentate
0 (dry seed)	7 out of 7	5 out of 8	20	83
1	6 ,, 8	0 ,, 8	17	0
2	0 ,, 8	1 ,, 8	11	1
3 (radicle emerging)	2 ,, 8	0 ,, 8	4	0
4	1 ,, 7	0 ,, 8	4	0
5	—	0 ,, 9	—	0
6	1 ,, 7	—	0	—
7 (cotyledons emerging)	2 ,, 8	0 ,, 8	0	0
8	4 ,, 8	0 ,, 8	0	0
	Comet	Potentate		
Nitrogen content	4·23 %	4·88 %	(air-dry basis)	
Wt. of 50 air-dry seeds	0·163 g.	0·146 g.		

The data presented in Table 3 may be interpreted in the light of Kausche's (1940) work with tobacco. During ripening and germination of tobacco seed, Kausche found that an inactivating substance was produced in both healthy and infected plants and this was held to account for the non-transmission of

tobacco mosaic virus through the seed. Even assuming that virus particles are confined to the outer surface of the seed coat in tomato seed, the production of an inactivator during germination would presumably be manifest by an apparent loss of virus activity in the crushed seed. If the seed and its parents develop under conditions of faulty nutrition, it may be reasonable to suppose that the production or efficacy of the inactivator will be limited, and such may have been the condition in the seed of 'Comet'. In this connexion it may be noted that the first experimental evidence of seed transmission in tomato, reported by Bewley & Corbett (1930), was by the germination of seed from *unripe* fruit.

In similar tests made on other batches of virus-infected seed the typical reaction was like that for 'Potentate', and of the samples tested none was found to react in a manner similar to 'Comet'. It may not be without significance that one of the first varieties to show symptoms of mosaic infection in Exp. II was this particular selection of 'Comet'.

The importance of environmental factors in determining the apparent resistance of plants to infection from internal or external sources can hardly be overemphasized and evidence of the influence of certain soil factors on symptom appearance will now be considered.

Experiment III, 1942

Influence of soil steaming on symptom appearance

The soil of the house used for Exp. II was steamed in the winter of 1941–2, and in 1942 variety trials were again laid down. Plots of the original 'Comet A' (seed from the same stock as that used for Exp. II) were included, together with plots of 'E.S.1' raised from virus-free seed. Each plot contained twenty plants and four plots of each variety were distributed evenly (not at random) over the house.

Observations were started at germination and mosaic symptoms did not appear until 6 June when the sixth truss was in bloom, despite the fact that no precautions against entry or spread of virus were taken. All the plants were extremely vigorous and of good colour during 1942. Exceptionally favourable weather conditions (daily mean no. of hr. bright sun, Apr.–June inclusive = 7·38) together with steam sterilization of the soil helped to produce a record crop in this season.

Under these conditions there was no evidence that plants raised from virus-free seed remained free from mosaic symptoms for a longer period than plants raised from infected seed, although all plants remained free from mosaic symptoms longer than in the preceding year. Steaming may have destroyed sources of infection in the soil in this experiment, but it is clear that, associated with the increased vigour of the plants, there was a definite tendency to resist infection via smoking tobacco, insects, or the seed, presuming one or more of these sources to have been efficient vectors. The vigour of plants, as related to potash manuring, has been shown to be associated with relative susceptibility to accidental infection with tomato mosaic virus (Selman, 1943).

Influence of soil texture on symptom appearance

In Exp. II plants showing symptoms of mosaic infection in the varieties 'Comet A' and 'Market King' wilted rapidly during periods of bright sunshine, and this was especially marked during the week following symptom appearance. This effect was noted earlier (Selman, 1941) with mosaic infection of crop plants, but it was far more severe on these two varieties than on plants in other parts of the house. Subsoil differences occur on different parts of the nursery and it was suspected that some local soil condition might be contributing to the onset of mosaic symptoms in certain parts of the house.

The topsoil of this house was a sandy, clay-loam, heavily manured and continuously cultivated for at least 20 years under glass. At a depth of approximately 18 in. is a silty-clay subsoil, which is much less permeable to water than the porous topsoil. A series of augur borings was made in the two borders to determine the depth of the subsoil and from these a rough north-south profile of the subsoil level was obtained.

In Fig. 5 the level of the subsoil is plotted together with the relative positions of the first few plants to show mosaic symptoms in the two borders in 1941 and 1942. There is a definite indication that mosaic symptoms tended to appear first on plants where the depth of topsoil was greatest. Further investigations are needed but this result is considered to be related to the greater fluctuations in water supply to roots confined largely to the porous topsoil. Tomato roots were not found below a depth of 19 in. in this house. Waterlogging does not occur under normal conditions when the crop is planted, although it has been noted in the winter at the north end of the house where the topsoil is shallowest.

Curtis (1940) found that plots of tobacco on coarse sandy soils showed a much higher degree of infection with tobacco mosaic virus than plots on fine sands or sandy loams. Favourable soil and moisture conditions were associated with a relatively low incidence of infection.

Conclusion

From this and earlier work there is evidence that apparent, 'delayed' seed transmission of mosaic-inducing viruses occurs in the tomato crop. This condition may be interpreted by postulating differences in the resistance of plants raised from seed of differing origin to the multiplication and systemic spread of those viruses, although whether the latter originate from sources internal or external to the plant is obscure.

Evidence from studies of the apparent virus content of germinated and ungerminated seed and of the embryo indicated that in certain circumstances, associated either with malnutrition or incomplete maturation of the seed, viruses may be present in the embryo and may persist in extracts of the germinating seed. Thus, there appears to be a strong probability that true, delayed seed transmission may sometimes occur.

Soil conditions controlling the effective functioning and development of the root system appear to exert a profound influence on the resistance of plants to systemic infection by relatively small numbers of virus particles, whether isolated internally, as would occur in delayed seed transmission, or whether introduced by external agents. Soil texture, water supply and the adequacy and balance of mineral nutrients are important factors in this connexion.

Where environmental conditions are exceptionally favourable to growth, plants raised from virus-free seed are unlikely to show advantage over other plants in respect to the incidence of mosaic infection. Such ideal conditions, particularly of the soil, can rarely be assured in commercial glasshouses, and where soil factors may tend to be unfavourable, plants raised from virus-free seed are likely to remain free from mosaic infection longer than other plants, even where precautions are not taken against the entry

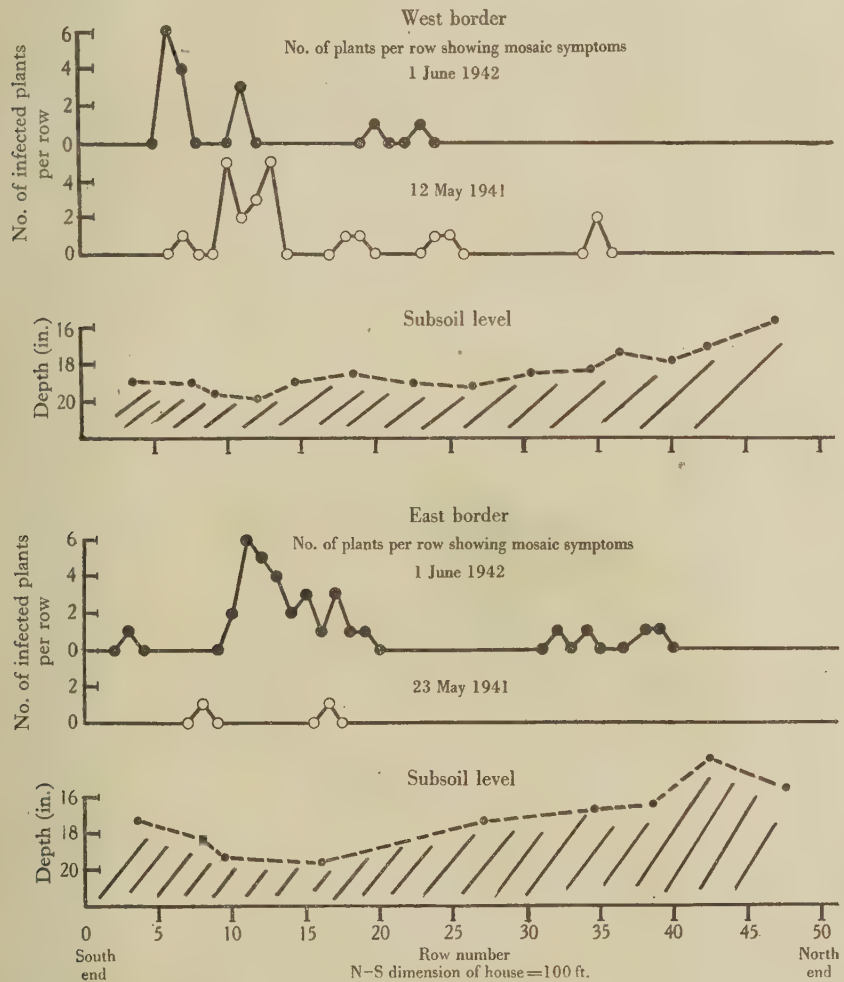


Fig. 5. Location of early infected plants and subsoil level.

and spread of mosaic-inducing viruses from other sources. On commercial nurseries, the use of virus-free seed taken from well-nourished, vigorous plants must be regarded as an essential prerequisite to the production of a virus-free crop.

The author is greatly indebted to Dr W. F. Bewley for much unpublished information and stimulating advice concerning the problem of seed transmission of viruses in the tomato crop.

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The reactions of potato varieties to viruses *X*, *A*, *B* and *C*

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(With Plate 6)

The reactions of 146 potato varieties to graft infection with viruses *X*, *A*, *B* and *C* are recorded, on the basis of their top-necrotic or non-necrotic symptoms. Evidence is given that the viruses *B* and *C* used in this work were uncontaminated with other viruses. Reference is made to the effects of varietal reaction on virus distribution.

A cardinal distinction between potato varieties in their responses to infection with viruses *X*, *A*, *B* and *C* is that some are killed with top necrosis whilst others produce non-necrotic symptoms. This distinction is based on simple genetic differences (Cockerham, 1937*a*, 1943; Cadman, 1942), and reason has been given for regarding a top-necrotic reaction as the index of virtual immunity from the causal virus under natural conditions of infection (Murphy, 1936; Cockerham, 1937*b*; Scott, 1938). Varieties which react in this fashion are now commonly described as field immune (Clinch, Loughnane & Murphy, 1938). The efficacy of field immunity and its practical significance, from the aspect of the distribution and control of viruses *X* and *A* in Scotland, was discussed by Cockerham (1939, 1940). There is, however, no comprehensive record of the reactions of potato varieties to viruses *X*, *A*, *B* and *C*, though the behaviour of a few varieties towards individual viruses has been described. A wide survey of potato varieties in their responses to infection with each of the four viruses was made and the results are given concisely in the appendix to this paper.

The varieties examined include most of those in

general cultivation and also a number which have little commercial value but which are of experimental and historical interest or of potential worth as parents in breeding for field-immune characteristics. For many uncommon varieties I have to thank Mr W. M. Findlay, Craibstone, Aberdeen, and Mr R. J. Scott, Department of Agriculture for Scotland, East Craigs, Edinburgh. I am also indebted to Mr K. Bėčius, Plant Breeding Station, Dotnuva, Lithuania, for a number of Continental varieties and to Dr K. M. Smith, Plant Virus Station, Cambridge, for a supply of U.S. seedling 41956.

The viruses used throughout the investigations were taken from single tested clones of the following varieties: virus *X* from Arran Victory, virus *A* from Irish Chieftain, virus *B* from Duke of York, and virus *C* from Edgecote Purple. It is believed that in these sources each virus was free from admixture with any other virus. Except where stated otherwise, the transfer of virus from these sources to the variety under test was brought about by graft unions. Occasionally, viruses present in the variety to be examined killed one or more of the varieties normally employed as infective scions, and to overcome this supplementary sources of infection were prepared by grafting several virus-free plants of Di Vernon with scions from the original sources of infection.

Symptoms of top necrosis, shown in the Appendix by the symbol N, were taken to indicate that infection of the variety under examination had been accomplished successfully. Acceptance of the virus without the production of top necrosis (symbol S) was confirmed in every instance by its recovery on a suitable indicator host, usually a potato variety which revealed its presence in the manifestation of top necrosis. The varieties Epicure for virus *X*, Great Scot for virus *A*, Arran Victory for virus *B* and Majestic for virus *C* were found to be most useful for this purpose.

The U.S. seedling 41956 falls outside the simple classification adopted for the majority of varieties as it failed to accept viruses *X* and *B* in a number of trials. The immunity of the seedling from these viruses is thus confirmed and is shown in the Appendix by the symbol I.

Virus *X*

The symptoms of top necrosis observed in response to infection with virus *X* were typically those described by Quanjer (1931) and Bawden (1932). The number of varieties reacting in this fashion were extended from six to twenty-nine, many of which have potential value as parents in breeding for field-immune characteristics. Field immunity is of particular value in seeking control of virus *X* since no other form of control is effective. The virus is disseminated by contact between diseased and healthy plants (Loughnane & Murphy, 1938 *a*, *b*). Consequently the spread of virus *X* is partly, if not entirely, independent of seasonal and ecological effects on aphis populations and it can take place in the well-established seed-producing areas where environmental advantages reduce the spread of aphis-borne viruses to a minimum (Clinch *et al.* 1938; Cockerham, 1939). Furthermore, the virus may be carried with little or no visible effects on some varieties, and control by roguing is extremely difficult.

Although the results of infection with virus *X* are not always visibly serious, it was shown by Cockerham (1937*a*) and Scott (1941) that yield may be reduced by 5–40% according to the variety affected and the particular strain of the virus concerned. These losses are relatively small when compared with those due to leaf roll and severe mosaic, and usually yield is not reduced to an uneconomic level, as it is with the severe diseases. Nevertheless, since over 70% of the total acreage of potatoes grown in Great Britain is planted with susceptible varieties the total annual loss in yield from virus *X* is considerable, for many varieties are completely permeated with the virus whilst most are infected to a large degree. These losses can scarcely be avoided so long as susceptible varieties preponderate in agricultural practice. There is no reason why they should preponderate in the future, for Cadman (1942) showed that field immunity from virus *X* is inherited as a Mendelian dominant and no insuperable difficulties have been found to stand in the way of breeding field-immune varieties. The essential genes are available within the range of varieties in cultivation and selective reassortment is all that is required for their employment to the greatest advantage. A possibility of great promise is thus open to the potato breeder, and in this respect the situation confronting him is similar to that which arose at the beginning of the present century when the control of wart disease raised an urgent problem. The discovery of varieties which were immune from wart disease then 'heralded a new era in variety production' (Salaman, 1937) which resulted in the raising of many of the most popular varieties in use to-day. The control of virus *X*, and of other viruses against which field immunity is effective, is equally urgent and calls for a similar response.

Virus *A*

Top necrosis due to virus *A* was described by Murphy & McKay (1932), Clinch & Loughnane (1933) and Bawden (1936). The disease differs from top necrosis due to virus *X* in its initial symptoms but not in its ultimate effect. On most necrotic reacting varieties symptoms first appear on the terminal leaves as a yellow blotchy mottle which rapidly gives place to large patches of necrotic tissue. At the same time lesions appear in the young tissues at the shoot apex. The spread of necroses from leaves to petioles and from the shoot apex to the main stem leads to the entire collapse of the affected shoot. A variant of this reaction was found on several varieties, one of which, Epicure, was reported by

Bawden (1936) to react in non-necrotic fashion to virus *A* but by Scott (1938) to react with apical top necrosis. Upwards of fifty infections of Epicure from sources known or suspected to contain virus *A* were made during the past few years, and invariably infection was followed by a necrotic collapse of tissue at the apices of affected shoots. Scott's observation is therefore confirmed. Symptoms appeared in 20–30 days from grafting and were first noticed as small necrotic spots immediately under the terminal buds (Pl. 6, fig. 1). Occasionally, no further necrosis took place and the extension of the shoot proceeded normally. More often the necrosis spread upwards into the terminal bud and brought about a typical but restricted form of top necrosis (Pl. 6, fig. 2). On Epicure there was no further development of symptoms on the aerial portion of the plant, but on some varieties, e.g. Great Scot, apical top necrosis was often followed by the appearance of blotchy necrotic patches on the leaves typical of virus *A* infection on most necrotic reacting varieties. Tubers from plants affected with apical top necrosis fall into three categories: fully necrotic, partially necrotic, or unblemished. Fully or partially necrotic tubers were either non-viable or produced small necrotic plants without vegetative progeny. Unblemished tubers gave rise to normal plants from which no virus could be recovered. In effect both apical top necrosis and full top necrosis are indicative of hypersensitiveness to virus *A* and hence of field immunity from this virus. No distinction has been drawn between them, therefore, in the table of varietal reactions.

Virus *A* is widely distributed and may in itself cause minor diseases and bring about reductions in yield (Scott, 1941). With virus *X* it is jointly responsible for the severe disease of crinkle (Murphy & McKay, 1932). The virus is aphis transmissible but is difficult to transfer by sap inoculation (Clinch & Loughnane, 1933). In consequence, its economic significance is confined chiefly to areas where aphides are abundant and, therefore, where the more severe diseases caused by the leaf roll virus and virus *Y* are of greater significance. In Great Britain upwards of 56% of the acreage under potatoes is planted with varieties which are field immune from virus *A*. There is reason to believe that the virus is of relatively small importance as a factor in disease causation in all but a few commercial varieties such as Golden Wonder, Catriona, and Immune Ashleaf which are known to be extensively, if not completely, infected with it. In these cases the problem of disease control arises chiefly from the infiltration of virus *X* into the stocks, thus leading to their degeneration, and not from the dissemination of virus *A* from them.

Virus *B*

Although virus *B* (Bawden, in Smith, 1933) is widely distributed throughout European, British, American, Andean and Australian potato varieties (Quanjer & Botjes, 1929; Bawden, 1936; Murphy & Loughnane, 1936; Dykstra, 1935, 1939; Dennis, 1939*a*; Bald & Pugsley, 1941) there is only one record of its occurrence in uncontaminated condition (Scott, 1938). Most authors agree that it is usually accompanied by virus *X*. When, therefore, a virus in Duke of York was found to cause top necrosis in Arran Victory and President but not in Epicure the possibility was examined that it was virus *B* alone. The absence of virus *X* in the Duke of York source was confirmed by making graft transfers to a number of varieties known to show top necrosis when infected with virus *X*. Some of these varieties, e.g. Craigs Defiance and Crusader, responded to infection with symptoms of top necrosis indistinguishable from those caused by virus *X* on the same varieties. The majority, including the more usual experimental varieties Arran Crest, Epicure and King Edward, showed only a fleeting *X*-type mottle. Scions from these latter induced top necrosis on Arran Victory and President and so confirmed the passage of the original virus to them. The identity of this virus with virus *B* was established by grafting scions from Duke of York to twenty-five of the varieties examined by Bawden (1936) for their reactions to virus *B* in Up-to-Date and which were known to give non-necrotic reactions when infected with virus *X*. Ten varieties reported by Bawden to give non-necrotic reactions with the Up-to-Date virus were also non-necrotic to the virus in Duke of York, and fifteen varieties which showed top necrosis when infected from Up-to-Date also showed top necrosis when infected from Duke of York. Such perfect agreement would be expected only if the viruses were identical, and it is concluded that the virus found in Duke of York was virus *B* uncontaminated with virus *X*. It follows that virus *B* does not cause top necrosis in Arran Crest, Epicure and King Edward, and the many reports of top necrosis in these varieties when infected from Up-to-Date sources are interpreted to indicate that virus *X* is a normal component of Up-to-Date 'streak'.

Dykstra (1935, 1939) and Dennis (1939*b*) claim to have separated virus *B* from virus *X* by removal of the latter from Up-to-Date 'streak' by 'filtration' through U.S. seedling 41956 which is immune from virus *X*. Attempts to repeat this work and so make possible a direct comparison between the Up-to-Date and the Duke of York viruses failed completely. No recovery of virus was made from 41956 grafted with Up-to-Date carrying 'streak' (*X*+*B*), or with Duke of York carrying virus *B* alone. The variety responded as though it were immune from both viruses. Bald & Pugsley (1941) and Clinch (1942) reported similarly. By double grafting, Clinch showed that both virus *X* and the entire virus content of Up-to-Date could travel passively and without change through large portions of 41956 stems though neither was recoverable from any portion of the plant by direct methods. In this connexion it is significant that Dennis (1939*b*) failed to recover either virus *X*

or virus *B* by sap inoculation from two plants of 41956 which had been grafted with Up-to-Date scions carrying these two viruses. His results are based on a single successful passage of virus from Up-to-Date to 41956 and from 41956 to *Datura* by graft. Since further transfers from *Datura* caused top necrosis on Arran Crest, Epicure and King Edward, it is clear that no separation of the two viruses had taken place. A similar check on Dykstra's (1935, 1939) claim to have separated the two viruses cannot be made as he gives no data for varieties showing top necrosis to virus *X*. He demonstrated that virus *B* could pass through 41956 by making double grafts with this variety as the intermediary between scions carrying *B*+*X* and stocks which were known to react with top necrosis to virus *B*. The latter succumbed with top necrosis thus indicating that virus *B* had entered them. His conclusion that virus *X* failed to pass was based on the negative results of inoculation from the necrotic basal stocks to *Datura* and pepper. It would seem that neither virus *B* nor virus *X* was present in these inocula, for sap inoculation from Duke of York with virus *B* to *Datura Stramonium* and *Capsicum annuum* have invariably evoked symptoms similar in type to those caused by virus *X* (Pl. 6, figs. 3, 4). It is possible that in Dykstra's experiments virus *X* was not filtered out but was merely restricted, with virus *B*, to the necrotic areas of the basal stocks. Similar restrictions of the two viruses were observed by Bawden (1936), Clinch (1942) and in this laboratory when sap inoculations were made from Up-to-Date plants carrying 'streak' to potato varieties which react with top necrosis to virus *B*. The usual result of such inoculation was the formation of local lesions at the site of infection. Most frequently these lesions were the only pathogenic effects and the remainder of the infected plants were virus-free. Occasionally the *X* component passed the barriers of necrotic tissue and produced systemic symptoms, and in such cases only virus *X* was recoverable from the infected plants.

The production of *X*-type symptoms on *Datura* and *Capsicum* by inoculation with virus *B* was repeatedly confirmed. It was found also that virus *B* produces similar symptoms to those caused by virus *X* on White Burley tobacco, *Nicotiana glutinosa*, Essex Wonder tomato, *Hyoscyamus niger* (Pl. 6, fig. 5) and *Solanum nodiflorum*. No symptoms were seen on nor was the virus recoverable from *Atropa Belladonna* and *Lycium barbarum*.

These results, together with the similarities in the types of top necrosis induced in many potato varieties and in the behaviour of seedling 41956, are suggestive of a close relationship between viruses *X* and *B*. As the serological identity of the two viruses has been established (Bawden, 1936; Dennis, 1939*b*; Clinch, 1942) and there is evidence of mutual protection between them (Clinch, 1942), the suggestion of the latter author that virus *B* is a strain of virus *X* appears to be well founded.

The distribution of virus *B* in Great Britain is closely linked with that of virus *X*, the two having been recorded in combination from most varieties which are susceptible to both. As yet there is no record of virus *B* from varieties which are field immune from virus *X*. The distribution of virus *B* is not as extensive as that of virus *X* but it occurs freely in varieties which are susceptible to both viruses, that is, in 55 % of the acreage of potatoes grown.

VIRUS *C*

A virus causing top necrosis in President but not in Arran Victory was found in Di Vernon by Salaman (1930) and in Monocraat by Quanjer (1931). Bawden (1936), working with virus from these sources, designated it virus *C*, and stated that in Di Vernon it was accompanied by viruses *X* and *Y* and in Monocraat by virus *X*. He gave the reactions of nineteen potato varieties to graft infection with the mixed viruses from these sources. With the exception of the reactions on Arran Banner and Arran Pilot Bawden's results were confirmed in the present instance with a virus which was first found in Edgecote Purple and later in Arran Rose, Cardinal, International Kidney, Liddesdale Lads, Kepplestone Kidney and Maud Meg. All these varieties react with top necrosis when infected with viruses *X* and *A* and hence are free from these viruses. Moreover, scions from them failed to induce top necrosis on Arran Victory from which it follows that they were also free from virus *B*. The possibility of virus *Y* being a contaminant was never disposed of with complete satisfaction. Upon varieties which are not killed the virus manifests itself in symptoms ranging from a vague chlorosis with slight leaf puckering and waving of the leaf margins as in Edgecote Purple, to severe mosaic with extreme distortion of the leaves as in Di Vernon and U.S. seedling 41956. These symptoms are typical of second year infection with virus *Y* in these varieties. The necrosis brought about by virus *C* from Edgecote Purple is also strongly reminiscent, in its early stages at least, of acropetal necrosis caused by virus *Y*. The resemblance is seen in the yellowing of the interveinal tissue in affected leaves and the appearance of minute lesions which coalesce to form streaks. The lower leaves, petioles and stems are affected as though with leaf-drop streak, but the apices and upper leaves show a typical top necrosis. The effect is thus far more severe than that of leaf-drop streak, and it results in the death of the whole affected stem including any tubers attached thereto (Pl. 6, figs. 6, 7).

Contrary to the experience of Bawden (1936), who worked with the needle only, and Dykstra (1939), infection through sap inoculation with carborundum as an abrasive was successfully achieved on several occasions. The potato vars. Epicure, Majestic, British Queen, Up-to-Date, Craigs Defiance and President, all of which show top necrosis when infected by

graft, each responded to sap inoculation with the formation of local necrotic lesions on the inoculated leaves (Pl. 6, fig. 8). With one exception, a single plant of Craigs Defiance which succumbed with top necrosis, no systemic invasion followed the production of these lesions. Arran Victory when infected in similar fashion, accepted the virus with the production of a chlorotic crinkle similar to that induced by virus *Y* on this variety. In this case the acceptance of virus *C* was confirmed by its recovery on Majestic upon which it causes top necrosis.

Sap infection of White Burley tobacco, *Nicotiana glutinosa*, Essex Wonder tomato and *Hyoscyamus niger* was followed in each case by the expression of vein-banding symptoms which were indistinguishable from those caused by virus *Y* (Pl. 6, fig. 9). On *Solanum nodiflorum* the symptoms were an interveinal mottle with necrotic streaks and dropping of the lower leaves, again similar to the effect of virus *Y*. Virus *C* was recovered on Majestic from the tomato plants included in each series of infections. No successful infection of *Capsicum annuum*, *Datura Stramonium* or *Atropa Belladonna* was accomplished.

The similarities of the effects of viruses *C* and *Y* on various hosts suggests that either virus *Y* is a constant contaminant of virus *C* or that the latter is so closely allied to virus *Y* that it evokes similar reactions on the part of the host. At present there are two lines of evidence which point to the individuality of virus *C* in Edgecote Purple and support the second of the above alternatives. As already stated, no systemic invasion followed sap inoculation of Epicure, Majestic, British Queen, Up-to-Date and President with sap from Edgecote Purple, yet sap inoculation of these varieties with virus *Y* from Craigs Defiance resulted in typical leaf-drop streak and severe mosaic. Secondly, attempts to transfer virus from Edgecote Purple to Arran Victory, Di Vernon, Majestic and tobacco by means of *Myzus persicae* failed repeatedly though parallel infections with virus *Y* from Craigs Defiance were largely successful. These attempts were made under various conditions of starvation of the aphides prior to feeding and for feeding periods varying from 2 min. to 6 days. It would seem that the virus in Edgecote Purple differs from virus *Y* in its necrotic effects on potato varieties and also in the mode of its transmission. The latter difference may be merely one of vector for three known instances of transmission in the field are recorded. Two of these relate to potato seedlings raised in 1939 and grown in the vicinity of but not contiguous with varieties infected with virus *C*. No symptoms of infection were observed in 1939, presumably the year of infection and a year of abnormally high aphis populations, but they appeared in the form of severe chlorotic mottles in the affected plants when these were grown under glass in the following year. Scions from these two plants induced top necrosis on Epicure, Up-to-Date and Majestic but not on Great Scot and Arran Victory. Further grafts to Edgecote Purple failed to induce top necrosis. These reactions indicate that viruses *X*, *A* and *B* were absent from the affected plants and that the mottling was due to virus *C*.

The third transmission was observed in 1941. In 1940 six virus-free plants of seedling 70 (13), upon which virus *C* is non-necrotic, and six plants of Craigs Defiance, upon which the virus causes top necrosis, were grown in a small bed in such a way that each plant was surrounded by *C*-carrying Edgecote Purple. All twelve plants retained a healthy appearance in 1940, but in 1941 a complete progeny of a single plant of 70 (13) showed a severe mottle which was ascribed to virus *C* as the result of graft transfers to Epicure, Great Scot, Arran Victory and Majestic. A repetition of this experiment in 1941, when aphis populations were considerably smaller, gave a negative result.

Although the disease induced by virus *C* is often severe there are few records of its occurrence. The virus has been found chiefly in British varieties which are old and uncommon, are field immune from viruses *X* and *A*, and highly tolerant towards virus *C*. Virus *C* is widespread, for it has been recorded in European varieties (Salaman, 1930; Quanjer, 1931; Bawden, 1936), in Andean varieties (Dennis, 1939*a*) and in a single old Australian variety (Bald & Pugsley, 1941), but not from any American variety (Dykstra, 1939). A possible explanation of the absence of American records for the virus is given in Dykstra's (1936, 1939) observations that all the American varieties tested succumbed with top necrosis when infected with virus *C* from European sources. This suggests that American varieties are protected from infection by field immunity. A similar reason may be advanced for the scarcity of the virus in Great Britain, for although only twenty-two of forty-three varieties listed by the Potato Marketing Board (1937) give a top-necrotic reaction when infected with virus *C*, these twenty-two varieties account for upwards of 77 % of the total acreage of potatoes planted.

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APPENDIX

The reaction of potato varieties to viruses X, A, B and C

I = no reaction (immune). N = top-necrotic reaction (field immune). S = non-necrotic reaction (field susceptible).

Variety	Viruses				Variety	Viruses			
	X	*A*	*B*	*C*		*X*	*A*	*B*	*C*
Aberdeen Favourite	S	N	S	S	Ben Lomond	S	N	N	S
Abundance	S	N	N	S	Bishop	S	N	S	N
Alannah	S	N	S	N	British Queen	S	N	N	N
Ally	S	S	S	N	Burnhouse Beauty	S	S	S	N
Alness	S	N	N	S	Cardinal	N	N	S	S
America	S	S	S	N	Catriona	S	S	N	S
Angus Leader	S	S	N	N	Celt	S	N	S	N
Argyll Favourite	S	S	N	S	Centifolia	S	S	S	N
Arran Banner	S	S	S	S	Champion	S	S	S	S
Arran Bard	S	S	S	S	Clar Innes	S	N	N	S
Arran Cairn	S	N	N	N	Claymore	S	N	N	S
Arran Chief	S	S	N	S	Colossal	S	N	S	N
Arran Comrade	S	S	N	S	Craigs Defiance	N	N	N	N
Arran Consul	S	S	S	N	Crusader	N	N	N	N
Arran Crest	N	N	S	S	Cumnock	S	N	N	S
Arran Luxury	S	S	N	S	Dargill Early	S	N	N	S
Arran Peak	S	N	S	S	Dean	N	N	S	N
Arran Pilot	S	S	N	S	Deodara	S	S	S	S
Arran Rose	N	N	N	S	Devanha	N	N	S	N
Arran Signet	S	S	S	N	Di Vernon	S	S	S	S
Arran Victory	S	S	N	S	Dominion	N	N	N	S
Asset	S	S	S	N	Doon Early	S	N	N	S
Ballydoon	S	N	N	N	Doon Pearl	S	N	N	S
Baron	S	N	S	S	Doon Star	S	N	S	N
Beauty of Bute	S	N	S	S	Duke of Perth	S	N	N	S
Benalt	N	N	N	N	Duke of York	S	N	S	N

Variety	Viruses X	A	B	C
Dunaverney	S	S	N	S
Dunbar Archer	S	N	N	S
Dunbar Cavalier	S	S	S	S
Dunbar Rover	S	N	S	N
Dunbar Standard	S	N	N	S
Dunbar Yeoman	S	N	N	N
Earlyfield	S	S	N	N
Early Market	N	N	S	N
Early Regent	N	N	S	N
Early Rose	S	S	S	N
East Neuk	S	N	N	N
Eclipse	S	N	S	N
Edgecote Purple	N	N	S	S
Edzell Blue	S	S	N	N
Entente Cordiale	S	S	S	N
Epicure	N	N	S	N
Evergood	S	S	S	N
Flourball	S	S	S	S
Gigantic	S	S	S	S
Gladstone	S	N	S	S
Golden Wonder	S	S	N	N
Great Scot	S	N	S	S
Harbinger	N	N	N	N
Herald	S	N	N	S
Immune Ashleaf	S	S	N	N
Imperia	S	S	S	S
International Kidney	N	N	S	S
Irish Chieftain	S	S	S	S
Irish Queen	S	N	N	N
Isis	N	N	S	N
Isla Star	S	S	S	N
Isle	S	N	N	S
Jubel	S	S	N	N
Kaiserkrone	S	S	S	S
Kalev	S	S	S	N
Kartz von Kameke	S	S	N	S
Katahdin	S	S	N	S
Katie Glover	S	S	N	N
Kepplestone Kidney	N	N	S	S
Kerr's Pink	S	N	N	S
King Edward	N	N	S	N
King George	S	S	S	N
K. of K.	N	N	S	S
Kunglia	S	N	N	N
Liddesdale Lads	N	N	S	S
Lochar	S	S	N	S

Variety	Viruses X	A	B	C
Lymm Gray	N	N	S	N
Macbeth's Castle	S	N	N	S
Main's Surprise	N	N	S	S
Majestic	S	S	S	N
Maud Meg	N	N	S	S
May Queen	S	S	N	S
Mein's Early Round	S	N	N	S
Mighty Atom	N	N	N	S
Myatt's Ashleaf	S	S	S	S
New Aberdonian	S	N	S	S
Ninetyfold	N	N	S	N
Nithsdale	S	N	N	N
Norna	S	S	S	N
Northern Star	S	S	N	S
Parnassia	S	S	S	S
Pepo	S	S	N	N
Perth Favourite	S	N	S	N
President	S	S	N	N
Puritan	S	S	N	N
Red Letter	S	N	N	S
Redskin	S	N	S	S
Rhoderick Dhu	S	N	N	S
Royal Kidney	S	S	S	N
Shamrock	S	S	S	N
Sharpe's Express	S	N	N	N
Sharpe's Victor	S	S	S	S
Shetland	S	S	N	S
Southesk	N	N	S	N
Spion Kop	S	N	N	S
Stärkereiche	S	S	N	N
Stirling Castle	N	N	S	N
Summit	S	N	S	N
Thorn I	S	S	N	S
Thorn II	N	N	N	N
Ulster Monarch	S	S	N	S
Up-to-Date	S	N	S	N
U.S. Seedling 41956	I	S	I	S
Utility	S	S	N	S
Vanguard	S	S	S	S
Warba	S	S	S	N
Waverley	N	N	S	N
Windsor Castle	S	S	N	N
Witchhill	S	S	S	N
Wohltmann	S	S	N	S
Wonderful	S	N	S	N
Yam	S	S	S	N

EXPLANATION OF PLATE 6

Fig. 1. Early stages in apical top necrosis on Epicure infected with virus *A*.

Fig. 2. Final stages in apical top necrosis on Epicure infected with virus *A*.

Fig. 3. *X*-type mottle on *Datura stramonium* infected with virus *B*.

Fig. 4. *X*-type local lesions on a leaf of *Capsicum annuum* inoculated with virus *B*.

Fig. 5. *X*-type mottle on a portion of a leaf of *Hyoscyamus niger* infected with virus *B*.

Fig. 6. Up-to-Date plant showing top necrosis with virus *C*.

Fig. 7. Cut tubers of Ballydoon (upper) and Pepo (lower) showing tuber necrosis with virus *C*.

Fig. 8. Local lesions on leaflet of Majestic inoculated with virus *C*.

Fig. 9. *Y*-type symptoms on *Hyoscyamus niger* infected with virus *C*.

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COCKERHAM—The reactions of potato varieties to viruses *X*, *A*, *B* and *C*

Studies on the spread of certain plant viruses in the field

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(With 2 Text-figures)

Studies on the spread of potato viruses *X* and *Y* and cucumber mosaic virus in the field are described: tobacco was used as the experimental plant. The plants were set out in the form of a cross, one series with the leaves in contact and one with the leaves not touching. No spread of potato virus *X* was observed, but there was extremely rapid permeation of virus *Y* throughout the plots. The spread of cucumber mosaic virus was much slower than that of virus *Y*.

Introductory

During the past few years field trials were carried out near Cambridge to investigate the natural methods of spread of potato virus *X* and to identify a possible insect vector. Throughout, tobacco was used as the chief test plant though the potato and *Datura Stramonium* were also employed. When the experiments were started, other viruses invariably appeared in the experimental plants and spread rapidly through the plots and, although some of these were new or undescribed, potato virus *Y* and cucumber mosaic virus appeared regularly every year. Since these viruses are aphis-borne, they were included in the experiments and their rate of spread compared with that of potato virus *X* which is not aphis-transmitted.

In preliminary experiments the test plants, potato, *D. Stramonium* and tobacco, var. White Burley, were grown in straight lines or concentric circles with the different species alternating. Certain of the potato plants contained potato virus *X* and were intended to serve as a source of infection for surrounding plants. In these experiments some of the plants, in contact with the source of virus (Smith, 1933), became infected with virus *X* but when the plants were spaced so that mechanical contact was impossible the infections ceased. Loughnane & Murphy (1938) showed that when healthy potato plants were grown in contact with a potato plant containing virus *X* in an insect-proof glasshouse, and a current of air was directed against the plants so that the leaves knocked together, the virus passed from the diseased to the healthy plants. They demonstrated that this occurred under field conditions and suggested that it was the natural mode of spread of the virus.

Methods

In the main experiments tobacco alone was used as a test plant, since it is the only species which reacts characteristically to infection with potato viruses *X* and *Y* and cucumber mosaic virus. The tobacco plants were set out in the field in two series of plots, each plot being in the form of a cross (see Figs. 1, 2). In the case of potato virus *X*, the plants in one plot were grown 4 ft. apart and were not in contact, and in the other plot they were only 1 ft. apart and were in contact. In each of these plots four *X*-infected tobacco plants at the centre acted as a source of virus. A duplicate series was set out to examine the spread of potato virus *Y* while a third series, similarly arranged but without a source of virus at the centre, was a control. There were, therefore, six plots in all. No plot was specially set aside for the cucumber mosaic virus since previous experience showed that this invariably made its appearance.

It was hoped that these experimental plots, with the plants growing under uniform conditions, would show whether potato virus *X* can spread when plants are not touching and, if so, how its dissemination about the plot compared with that of the two aphis-borne viruses. Further, it was hoped that the results would show whether the latter could also be spread by mechanical contact between diseased and healthy plants.

The experimental plants were set out in the field as soon as the danger of frost was over, i.e. 18 May. When the plants were established and as soon as the leaves of adjacent plants were touching in the 'contact' plots (25 June), the infected plants to serve as sources of virus were set out.

Discussion and results

Potato virus X

So far as the spread of virus *X* is concerned the results were negative, no infections with this virus being observed either in the 'spaced' or 'contact' plots.

Since potato virus *X* does not generally become systemic in tobacco plants above a certain size, the criticism may be made that it would be impossible for a non-systemic virus to spread from plant to plant by contact. To meet this the two plots were duplicated in midsummer and the sources of virus infection and the experimental plants were set out in the field at the same time, small plants about 3 in. across being used as these would easily become

systemically infected. In this experiment also, no spread of virus *X* in either plot was observed.

To test once more whether virus *X* could spread by contact among tobacco plants under controlled conditions, an experiment like that of Loughnane & Murphy was done in the glasshouse. A tobacco plant infected with virus *X* was placed in contact with a row of healthy tobacco plants, each with its leaves in contact with its neighbour and a current of air from an electric fan was directed across the plants. In two such experiments the plants next to the source of infection on either side became diseased but no further infection passed down the row. This shows that potato virus *X* can be passed from diseased to healthy tobacco plants by mechanical contact though it may be suggested that this more easily transmits it in the potato. The virus may, however, pass more easily under the controlled conditions of the glasshouse than in the field.

Potato virus Y

With the aphis-transmitted viruses the conditions are very different. Figs. 1 and 2 show the progressive infections of the tobacco plants by potato virus *Y*, the observations being made weekly. They illustrate the general plan of the plots, the numbers of plants in each plot, and the details of times of infection. In the spaced plot (Fig. 1) seventeen out of the forty plants were infected in less than a month after the four virus-infected plants, the source of infection, were set out in the field: a week later, 28 July, every plant was infected. Fig. 2, representing the 'contact' plot with 160 plants, gives details of the numbers of plants infected after each weekly recording. About 4 weeks after the sources of infection were put out in the field, sixty-three plants out of the 160 were infected: a week later seventy-nine more were infected, i.e. 142 out of the total of 160. When the experiment was discontinued at the end of August only four plants remained healthy. Such an extremely rapid and complete distribution of the virus throughout the plots is rather surprising. The data make possible certain tentative conclusions. Clearly some at least of the winged aphides arriving in the plot were already infected with the virus as certain plants which showed the disease first, were farthest from the source of virus at the centre of the plot. The order of the infected plants suggests that the movement of the aphides within the crop is largely among adjacent plants rather than to plants some distance away. Lastly, there is no evidence that potato virus *Y* spreads by mechanical contact, as well as by aphis, at all events in tobacco, since it

Fig. 1. Diagram of 'spaced' plot with plants not in contact, showing dates when each plant became infected with potato virus *Y*.

was common during the experiments to have healthy plants between two diseased neighbours.

Spread of potato virus Y in the control plots

On 20 July observation showed that there were two infected plants in the 'spaced' control plot which had no source of infection compared to seventeen in the 'spaced' plot with sources of virus infection at the centre. On the same date the contact control plot showed seven infections compared with sixty-three in the contact experimental plot. Thus, it seems clear that *close* proximity of a source of virus infection, as represented by the four diseased plants at the centre of each experimental plot, was an important factor in the spread of the disease. At the end of the experiments (18 Aug.) the spaced control plot showed thirty-two diseased plants out of sixty-four, and the 'contact' control plot sixty-two diseased plants out of 160, compared with practically 100% infection in the experimental plots. If the experiments had been prolonged the control plots would probably have shown 100% infection by the end of the season.

Spread of cucumber mosaic virus

The third virus under observation, cucumber mosaic virus, reached the plots considerably after the appearance of potato virus *Y*, presumably because it had to pass a greater distance, there being no known source of infection in the immediate vicinity. Furthermore, once it was present in the plots, its subsequent spread among the plants was much slower than that of potato virus *Y*. Although *Myzus persicae* is the vector of both viruses evidence suggests that it transmits potato virus *Y* more easily and efficiently than the cucumber virus. The final counts of tobacco plants infected with this virus were made on 21 Sept. about a month after the last recording of infections with potato virus *Y*. Since no plots were set aside expressly for cucumber

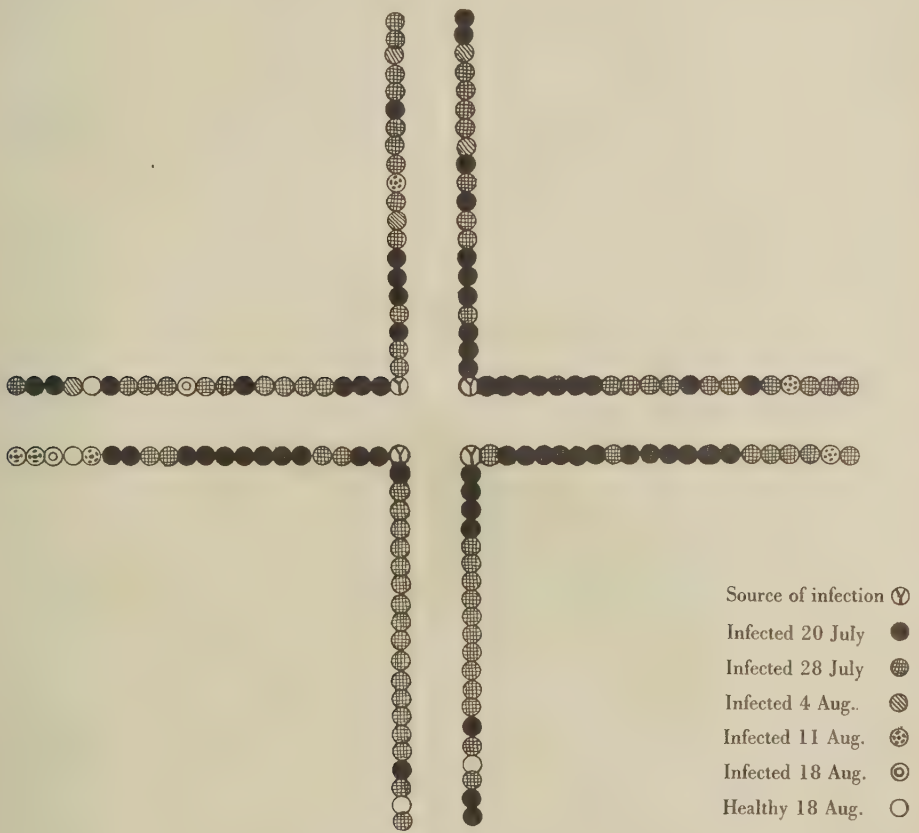

Fig. 2. Diagram of 'contact' plot with plants touching, showing dates when each plant became infected with potato virus *Y*.

mosaic virus, observations were made on the two control plots and the two plots used in the experiments with potato virus *Y*. The numbers of plants infected with the cucumber mosaic virus were as follows: control spaced plot, 10 plants; control contact plot, 9 plants; experimental spaced plot, 11 plants; and experimental contact plot, 15 plants. These numbers are much lower than in the case of potato virus *Y*. The positions of the plants infected with cucumber mosaic virus in the plots indicate that as with potato virus *Y*, transmission by mechanical contact does not take place in tobacco.

The writer is indebted to Dr W. D. MacClement for his assistance in carrying out the experiments.

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Further investigations on the biology and control of the carrot fly (*Psila rosae* F.)

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(With 3 Text-figures)

Records were obtained for the emergence of the first and second generation flies at Chatteris and Cambridge. There is a correlation between the age of the carrot crop and the commencement of the emergence of the second generation from it. The first generation was successfully controlled by killing the flies with a poison bait sprayed on the dikesides of the carrot fields. This treatment reduced the population of second generation flies and also their attack. Spraying the dikesides and/or the headlands of eight maincrop carrot fields in August and September greatly reduced the second generation damage. Very high kills of carrot fly were obtained on the headlands of treated fields. Poor results were given by the treatment on three very weedy fields which were incompletely treated. A marked increase in attack occurred in three untreated areas in 1942 as compared with the previous year.

Application of the poison bait spray in the field is discussed. Creosote-treated string gave promising results in small-scale experiments for the control of the second generation. Calomel dust (4%) with and without grass cuttings caused no reduction in attack.

In hot sunny weather the flies shelter in the vegetation around carrot fields from about 10 a.m. until about 5 p.m. In the cooler conditions of early morning and evening there is a movement of flies into the field. This rhythm is not maintained in dull warm weather.

Seedling carrots may be attacked in the field any time after the cotyledon stage. This enables the first generation of flies to propagate on main crop carrots. Soil sections about carrot rows showed that pupae of the overwintering generation are closely congregated around the carrots. Various insecticides were tested for the destruction of pupae in the soil. A creosote-sawdust mixture gave the best results.

Storage experiments showed that clamped carrots deteriorate much less than those unlifted. The population of flies is greatly increased by allowing attacked carrots to remain unlifted throughout the winter or by ploughing in.

In a previous communication (Petherbridge *et al.* 1942), observations and experiments on the biology and control of the carrot fly were described, and the following account deals with the continuation of these observations. The poison bait treatment worked out in 1941 was again tested on a field scale, being used with success on 174 acres of maincrop carrots, and 23 acres of early carrots. This method appears to be satisfactory for the control of carrot fly on a commercial scale.

EMERGENCE OF CARROT FLY, 1942

A detailed knowledge of the period during which the carrot flies emerge is necessary for the correct timing of the application of poison bait, and this was obtained by following the emergences in muslin cages set up over unlifted carrots and carrot clamp sites. Barnes (1942) showed that there is a considerable variation in the emergence period from year to year. From unlifted carrots at Hall Farm, Mepal, Isle of Ely, flies of the first or over-wintering generation were emerging from 11 May until late July, the highest rate of emergence being in the last 2 weeks of May. Similar results were obtained from hemlock (*Conium maculatum*), and a clamp site, in the same locality. From unlifted carrots at Cambridge, emergence lasted from 8 May until the end of June, with the highest rate in mid-May.

There is a marked correlation between the age of a carrot crop and the date that emergence of the summer generation begins. From early carrots sown in late March, emergence started on 20 July; from sowings made 13 Apr., the first flies were obtained on 4 Aug., and from carrots sown 27 May, flies did not emerge until the end of August. In all cases emergence continued until the second week in October (see Figs. 1 and 3).

The relation apparently depends on the length of the emergence period of the first generation (normally more than 2 months) and the date at which the crop is normally first susceptible to attack, i.e. in the first and second rough leaf stages. Early carrots sown during late March or early April will hence be liable to attack by the whole of the first generation of flies; maincrop carrots sown in late May, and reaching the susceptible stage about one month later, will be liable to attack by the second half only of the first generation. June-sown carrots will escape attack from all but the few flies present in July. Although variations in weather conditions during germination and soil conditions can affect the rate at which carrots reach the first and second rough leaf stage, as a general rule sowings made later than April become susceptible to attack after about one month. Since the minimum time for egg-to-adult development was found to be 2 months, the relation between date of sowing and date of emergence of the second generation flies is explained. In the Chatteris area, it has also been found that the later the date of sowing, the smaller the population of second generation flies appearing from that crop.

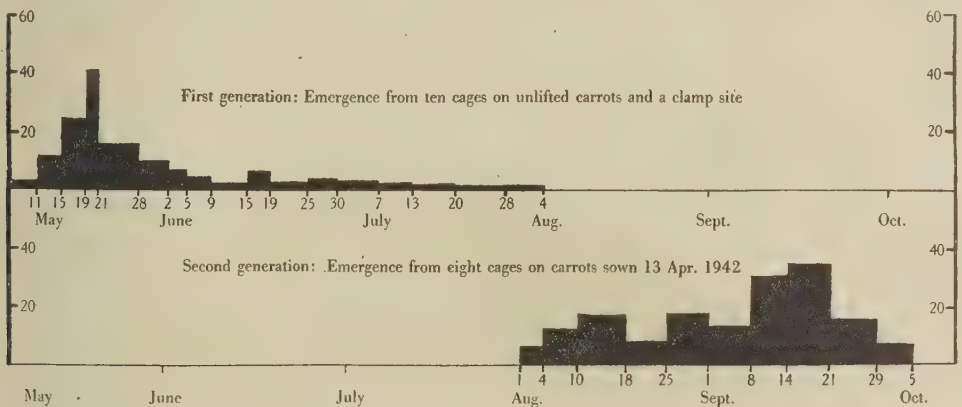
First generation: Emergence from ten cages on unlifted carrots and a clamp site
60
40
20
11 15 19 21 28 2 5 9 15 19 25 30 7 13 20 28 4
May June July Aug. Sept. Oct.
Second generation: Emergence from eight cages on carrots sown 13 Apr. 1942
1 4 10 18 25 1 8 14 21 29 5
May June July Aug. Sept. Oct.

Fig. 1. Diagram showing the emergence of carrot fly in 1942. Vertical scale in numbers of flies emerging per day. Area of diagram proportional to actual numbers of flies recorded.

EXPERIMENTS ON FIELD SCALE CONTROL OF CARROT FLY, 1942

Control of the first generation, 1942

To control the first generation, the measures used were those which had proved successful against the second generation in 1941. These consisted of spraying the dykesides or hedgesides around the carrot fields with a coarse spray of poison bait containing 2·5% molasses and 0·8% sodium fluoride.

Of eight fields of early carrots at Benson's Farm, Chatteris, three fields (totalling 23 acres) were treated. Flies were first swept in this locality on 19 May; six treatments were then given at 3–6 day intervals from 22 May until 12 June.

Spraying was confined to the dyke regions, whence 90% of the flies could be swept: the headlands were left unsprayed. Further sweeping carried out on the fields showed large decreases in carrot fly population consequent on treatment, and many dead flies were found on the treated fields.

At New Road, Chatteris, two adjacent carrot fields, each of 6 acres, were similar in all important respects —soil, previous cropping, dykeside vegetation and size of carrot crops—and therefore particularly suitable for comparison. One field was treated, and the other left untreated. Fig. 2 shows the variations throughout the season in the numbers of flies swept

from each field. The fly population of both fields was very similar before treatment started. Over the whole period of treatment, however, far less flies occurred on the treated field than on the untreated field, especially during the third to sixth sprayings, which, in contrast to the first two, were done in favourable weather.

Carrot-fly damage first became evident in late June; the headlands of the untreated field (at New Road) contained many patches of collapsed tops, caused by severe root attack. Many of these plants died, and rot prevented their inclusion in future samples. Similar damage occurred in another untreated field, but not in *any* treated field.

Carrot samples were taken during June, July and August from the headlands and midfield regions of the eight fields. Table 1 shows figures for the damage at New Road.

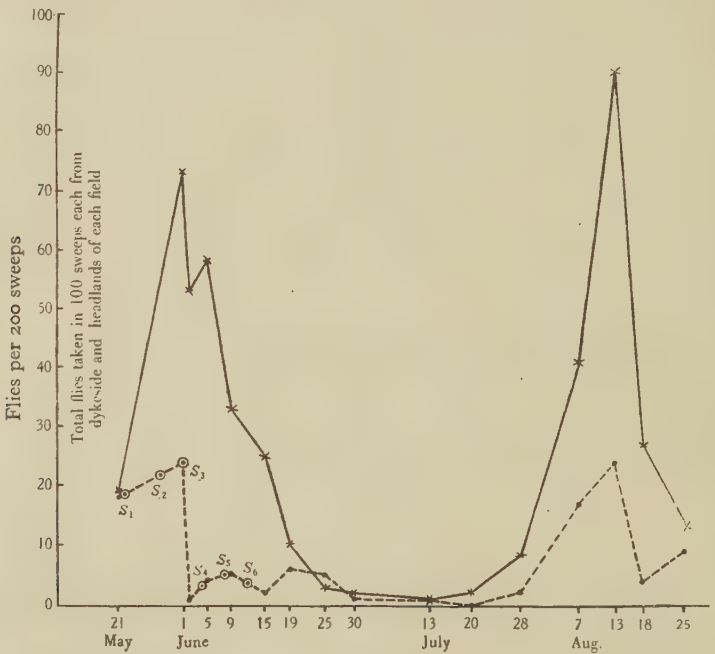

Fig. 2. Abundance of carrot fly on early carrots at New Road, Chatteris, 1942.
×———× Untreated field. ●– – – –● Treated field.
⊙ S_{1-6} denotes application of poison bait spray. On 1 June sweeping was carried out *before* spraying.

TABLE 1. *Control of carrot fly on early carrots. New Road, Chatteris*, 1942

		Treated field			Untreated field		
Date of sampling	Location of sample	Carrots clean %	Carrots unsaleable %	Total mines per 100 carrots	Carrots clean %	Carrots unsaleable %	Total mines per 100 carrots
25 June	4 headlands	98	0	4	84	4	24
15 July	,,	98	0	2	76	10	44
20 Aug.	,,	92	0	8	48	16	94
25 June	Midfield	100	0	0	98	0	2
15 July	,,	100	0	0	100	0	0
20 Aug.	,,	94	0	6	90	2	18
4 Aug.	S. headlands	97	0	4	74	2	40
20 Aug.	,,	86	0	19	57	2	68
8 Sept.	,,	55	0	61	40	8	141
25 Sept.	,,	50	0	105	22	13	259
28 Oct.	,,	30	2	161	10	20	355
16 Nov.	,,	24	6	205	3	32	491

Samples taken from the four headlands and from midfield each consisted of 50 carrots. Samples taken from the south headlands each consisted of 100 carrots. All samples taken at random.

At Benson's Farm in late July, the attack averaged 3% on the headlands and 1% in midfield for the untreated fields: no attacked carrots were found on the two treated fields at this date.

The effect of controlling the first generation on the abundance of the second generation, and its attack

Emergence of the second generation from early carrots at New Road, Benson's Farm, began on 20 July. Thenceforward, until the end of August, when the crops were lifted, the two fields were swept as for the first generation. The data obtained are shown in Fig. 2. In all parts of the treated field there were fewer second generation flies than on the untreated fields.

The effect of this reduction in fly population on the intensity of maggot attack was shown by sampling the two south headlands (unlifted by request) throughout the autumn. The figures obtained are given in the second part of Table 1.

In spite of the probable migrations of flies from field to field, first generation control alone had a pronounced effect on the total attack in the field.

Control of the second generation, 1942

Field scale treatments for the control of the second generation on maincrop carrots were carried out at Risby (West Suffolk), Chatteris (Isle of Ely), and Mepal (near Chatteris). Poison bait spray was applied as in 1941.

The Risby centre was selected for treatment because investigations at one farm (Quay's Farm) in 1941 had shown maggot damage to be fairly heavy. All the fields on this farm, adjoining Risby village, were treated, and also other carrot fields within a radius of 1½ miles of the village. This plan covered eight fields, four east of Risby on Quay's Farm (totalling 110 acres), one 18-acre field west of the village, and three fields (totalling 24 acres and of approximately equal size) to the south, across the main Newmarket-Bury St Edmunds road. All the fields were sown at the end of April or in early May.

The hedgesides of these fields were swept for carrot fly at the end of May, and flies were common on all of them. The development of the carrots then varied from the cotyledon stage in some fields to one and two rough leaves in others. Small numbers of flies were swept in June and July, very few being present at the end of July. At the end of the first week in August, second generation flies increased greatly in numbers on these fields. Spraying commenced on 11 Aug. and was repeated eleven times at 4-day intervals (last application 21 Sept.). During that period fewer flies were swept at any one time than immediately before spraying. At Quay's Farm the vegetation of the banks and lower hedge only was treated, the headlands being fallow. On the other four fields carrots were sown on the headlands which, with two exceptions, were also sprayed.

Sampling began on 16 Oct. and showed that the eight fields fell into two categories. One comprised the four fields at Quay's Farm, plus the 18 acres west of Risby: there the attack was very slight, and averaged only 5·7% in midfield, with 7 mines per 100 carrots. On the other group of three fields the attack was moderately heavy, averaging 33·3% in midfield, with 53 mines per 100 carrots. A second sampling was carried out on 4 Dec. On the first group of five fields, little further deterioration had occurred, but on the other three treated fields deterioration was very marked, the attack now averaging 60% in midfield with 137 mines per 100 carrots.

Further sampling showed that the average midfield infestation on these three fields was about as heavy as that on the headlands. Experience from sampling other fields in previous years and the Quay's Farm group in 1942, showed this to be a very abnormal distribution of attack, probably resulting from an abnormal distribution of flies. Sweeping has shown the distribution of flies to be largely governed by the available shelter, in which the flies tend to congregate. The three fields in which the treatment had apparently failed were full of weeds during July and August (due to labour shortage), a vigorous growth of *Chenopodium album* (fathen) and *Solanum nigrum* (black nightshade), providing excellent shelter over the whole of the fields. It is extremely probable that many of the flies sheltered in this vegetation instead of that on the headlands and hedge-banks; therefore only a small proportion would be killed by spraying the perimeters of the fields. This would appear to be the chief reason for the apparent inefficacy of the treatment on these three fields. A second complicating factor was that one headland and adjoining hedgeside in each of two fields were not sprayed. Independent sampling of these two headlands showed them to be more heavily attacked than the three treated headlands in each field.

Finally, a field of carrot seed adjoined one carrot field and was separated from a second by a narrow drove and low hedge. The third field, some distance from the carrot seed, was the least heavily attacked of the three, but all were about equally weedy.

Sampling across the first field, to give a transect, showed a big decrease in attack from the headland adjoining the carrot seed (unsprayed) to the opposite headland (sprayed). The discrepancy between the two headlands was much greater than that observed between sprayed and unsprayed headlands in the third field where there was no extraneous factor such as carrot seed. The carrot seed certainly provided a fruitful source of first generation flies in May and June, and later probably gave rise to a second generation. In these three fields, therefore, the factors of shelter in the field, a nearby source of carrot fly and incomplete spraying, greatly decreased the effect of the treatments.

Disturbing factors such as the above were not

encountered in the Quay's Farm group of fields where, as in the previous year, the crops were particularly free from weeds. Table 2 shows that the resulting infestation on this farm was lower than in the previous year, 1941.

A comparison of the infestation in 1941–2 was also made for three other areas (see Table 2). At Swaffham (Norfolk), carrots were grown extensively for the past few years on several adjoining farms. Parsnips, another host of carrot fly, are also frequently grown there. The slight damage found in 1941 had greatly increased in 1942: from the headlands of one field as much as 32% unsaleability was recorded. In north-west Norfolk the attack was heavier in 1942, but the increase was smaller than at Swaffham, probably because carrots are taken less frequently in the rotation and the fields are scattered. At Mepal a marked increase in attack was recorded for 1942.

At Hall Farm, Mepal, there were two part fields of carrots, each of 4 acres, about 300 yd. distant from each other. On an adjoining farm were two carrot fields (12 and 9 acres) about 500 yd. apart. These four fields were sampled before treatment to find the extent of the attack by the larvae produced by the later emerging flies of the first generation. On one field of each pair the attack was more than twice as heavy as on the other. The fields chosen for treatment were those most heavily attacked; it was expected, therefore, that at least twice as many second generation flies would appear on each of the fields to be treated as on the other member of the pair. The dikeside and crop shelter around the 12-acre (treated) and 9-acre (untreated) fields were very similar. Around the 4 acres to be treated, there was an abundance of suitable shelter consisting of potatoes on both the long headlands, and a hedge and a reed-filled dike on the other two. The second

TABLE 2. *Comparison of carrot-fly infestation at Risby (west Suffolk), Swaffham (Norfolk), north-west Norfolk, and Cavenham (west Suffolk) in the years 1941 and 1942. All samples midfield*

Locality	Date of sampling	Acres sampled	Carrots clean %	Carrots unsaleable %	Total mines per 100 carrots
Swaffham	2. x. 41	218	99·8	0	0·2
	13. xi. 42	182	78·4	0	36·6
North-west Norfolk	29. ix. 41	220	99·3	0	0·7
	20. x. 42	159	91·8	0	12·2
Mepal (Isle of Ely)	7. xi. 41	22	79·5	2	27
	9. xi. 42	21	65	1·2	57·5
Risby, Quay's Farm	9. xii. 41	114	84·5	1·75	27·5
	4. xii. 42	70	89·7	0	12·0 (treated)
Cavenham (nr Risby)	16. x. 42	105	79·5	0·3	30
Heath Barn (nr Risby)	4. xii. 42	25	59	7	97·0

Four fields of maincrop carrots were sampled at Cavenham (2½ miles north of Risby village), and a fairly heavy infestation was recorded in mid-October. Samples were also taken from a 25-acre field about one mile north of Risby, which was not sprayed on account of its windswept position. In December, the attack on this field was heavy (see Table 2).

These sampling records show that at Risby in 1942 the Quay's Farm group of five fields was far less heavily attacked than any other fields in the vicinity. Furthermore, this was the only area sampled where the attack had decreased in 1942 as compared with 1941; in all others there was a marked increase in attack in 1942.

Control of the second generation at Chatteris and Mepal (Isle of Ely), 1942

For the control of the second or summer generation of carrot fly, three fields of carrots on fen soil in the Isle of Ely were selected for treatment. Two of the fields (4 and 12 acres) were at Mepal, and the third (30 acres) at Horseway, Chatteris. The carrots were sown in the latter half of May.

4 acres (untreated) was bounded by two dikes almost free of tall vegetation, celery, and a drove, all of which provided comparatively little shelter for flies.

Each field was sprayed fifteen times, starting on 8 Aug. and ending 4 Oct. The interval between each application was 4 days, reduced to 3 days between 30 Aug. and 16 Sept. Fig. 3 shows the abundance of the carrot flies obtained by sweeping on the east and west headlands of a treated and untreated field at Mepal.

The first six sprayings were applied before flies became common on either field and were therefore largely wasted. At this time the connexion between date of sowing and the time of appearance of the second generation flies was not fully realized (see paragraph above on emergence of the second generation). Spraying was therefore commenced as soon as the second generation appeared in numbers on early carrots. The changes in the abundance of flies on the other pair of fields at Mepal followed a very similar course. In each of the treated fields the general level of abundance of flies was considerably lower than in the untreated fields.

The effectiveness of the spray was also measured by the number of dead flies found on the days following treatment. Sections 1 yd. wide and 4 yd. long were taken at random across the headlands, and the numbers of dead carrot flies thereon counted. The data obtained, together with an estimate of the numbers of flies killed on the perimeter of the 4-acre field, are shown in Table 3. Similar very high kills were obtained on the 12-acre field. Four transects made on two occasions across the headlands of the untreated 4 acres yielded a total of five dead carrot flies.

Both pairs of fields were sampled for maggot damage at intervals until early winter. A comparison of the deterioration in the midfield regions of the 4-acre fields is shown in Table 4.

Samples taken over the same period from the headlands of these fields show even greater differences: on the treated field the damaged carrots increased by 32% and the number of mines per 100 carrots from 92 to 206, the corresponding figures for the

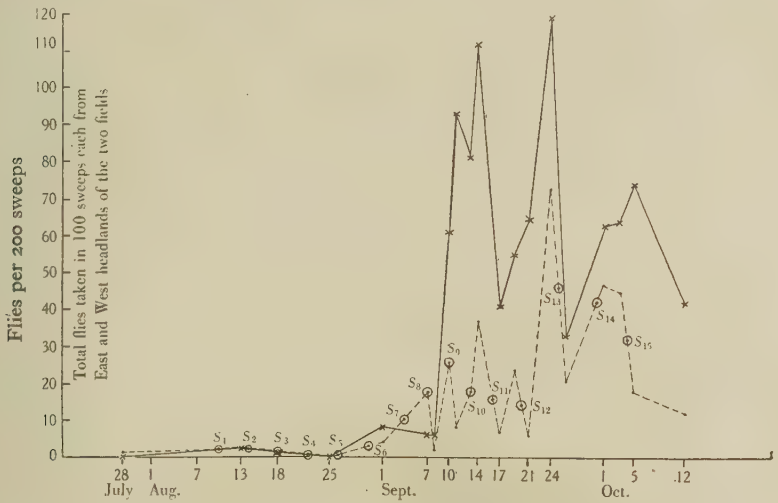

Fig. 3. Abundance of second generation of carrot fly on treated (12 acre) and untreated (9 acre) fields at Mepal, 1942.

×——× Untreated field. ●- - - -● Treated field.

⊙ S_{1-15} denotes application of poison bait spray.
Sweeping was carried out *before* spraying, when both were done on the same day.

TABLE 3. *Kill of carrot fly by poison bait. Hall Farm, four acres—treated* 1942

Total length of four headlands = 644 yd. Samples taken on headlands across rows 6–16, and 1 yd. wide.

Date of counting	Date of application of last spray	No. of dead carrot flies on 1 × 4 yd. strip	Estimated kill on total headlands, all round field
11 Sept.	10 Sept.	177 (E. headland, 3 samples)	106,200
14 Sept.	13 Sept.	210 (W. headland, 2 samples)	128,000
17 Sept.	16 Sept.	291 (E. headland, 2 samples)	187,726
26 Sept.	25 Sept.	175 (E. headland, 1 sample)	112,700
5 Oct.	4 Oct.	113 (E. headland, 1 sample)	72,772

1 Sept. and 1 Oct.: Dead carrot flies present in fair numbers. Spraying took place on 30 Aug. and 30 Sept.

TABLE 4. *Deterioration on treated and untreated four acres. Hall Farm, Mepal,* 1942, *midfield samples*

Date of sampling	Clean %	Unsaleable %	Total mines per 100 carrots
(i) Treated field. Four acres			
10 Aug.	60	0	48
8 Sept.	78	0	30
8 Oct.	78	0	24
26 Oct.	76	0	38
9 Nov.	42	0	104
25 Nov.	40	0	134
9 Dec.	44	0	102
28 Dec.	34	6	166
(ii) Untreated field. Four acres			
10 Aug.	82	2	24
8 Sept.	68	0	60
8 Oct.	70	0	38
26 Oct.	70	4	60
9 Nov.	48	6	130
25 Nov.	26	12	234
9 Dec.	34	12	194
28 Dec.	10	26	340

untreated field being 78% increase in attacked carrots, while the number of mines per 100 carrots rose from 18 to 432.

On the other pair of fields at Mepal, the damage was much less. Samples taken in both fields from 13 Aug. to 26 Oct. showed no measurable increase in the midfield attack on the treated (12 acres) field, while on the headlands the mines per 100 carrots increased only from 18 to 26. On the untreated (9 acres) field, the increase in mines per 100 carrots over the same period was from 0 to 14 and 8 to 58, for the midfield and headland regions respectively.

A comparison of the attack on the treated (12 acres) field with that on four untreated fields in the same locality is shown in Table 5.

TABLE 5. *Control of carrot fly. Second generation, 1942. Comparison of attack on one treated and four untreated fields. Mepal, nr Chatteris. Samples taken 26 Oct.–9 Nov.*

	Treated field (12 acres)		
Sample	Clean %	Unsaleable %	Total mines per 100 carrots
Midfield	96	0	4
Headlands	80	0	26

	Four untreated fields (average)		
Sample	Clean %	Unsaleable %	Total mines per 100 carrots
Midfield	65	1·0	57·5
Headlands	53	4·0	85·5

TABLE 6. *Control of carrot fly. Second generation, 1942. Comparison of attack on one treated and three untreated fields. Horseway, Chatteris. Samples taken 30 Nov. 1942*

	Treated field		
Sample	Clean %	Unsaleable %	Total mines per 100 carrots
Midfield	95	0	6
Headlands	89	0	13

	Three untreated fields (average)		
Sample	Clean %	Unsaleable %	Total mines per 100 carrots
Midfield	70·3	0·3	49·0
Headlands	59·3	3·7	88·3

Taking into account the initial attack on the two pairs of fields at Mepal, had no treatment been carried out, then it is almost certain that the attack on the 12 acres and 4 acres (both treated) would have been much heavier than on the other members of the two pairs.

A 30-acre field of maincrop carrots at Horseway, Chatteris, also received the same treatment as those at Mepal. Nearby were three untreated fields (8, 27 and 30 acres) with carrots of similar age. The dike-side vegetation and other features of fly shelter were similar for all four fields. A comparison of the attack on the headlands and midfield regions of the four fields (on 30 Nov. 1942), is shown in Table 6. The difference in the attack on the treated and untreated fields approximates to that obtained at Mepal, as shown in Table 5.

Sprays and spray applications

The spray used in all field experiments in 1942 contained 2·5% cane molasses and 0·8% powdered sodium fluoride. Beet molasses were not used as continental workers claim comparatively poor results when it is used in sprays against the beet fly (*Pegomyia betae*). Tests carried out under laboratory conditions with the carrot fly showed, however, that sprays made up with both beet and cane molasses gave an equally rapid and complete kill.

All applications were made by a mobile power-operated machine delivering spray through coarse nozzles at high pressure. Hand-operated knapsack-type machines were found to be too slow and laborious for field-scale work, and their spray gave a comparatively poor penetration of the vegetation.

For both the first and second generations the spray was applied to both sides of the dike or to the bank or lower part of the hedge surrounding the crop. Vegetation up to bush height, growing around or in pits in the fields was well treated, nettle beds receiving special attention. Where the carrots adjoined potatoes, the outer three or four rows of this crop were also treated. It was found that three nozzles on movable lances could effect this treatment satisfactorily. For control of the first generation it was found unnecessary to spray the headlands of the fields. For the second generation the headlands, where possible, were also sprayed. To do this a 9 ft. boom, carrying 11 nozzles, was attached behind the machine. This device covered a 4 yd. strip round the headlands of the field with a moderately coarse spray and gave a more even cover than the use of movable lances. When shelter disappeared, as by the cleaning out of a dike, or the death of potato foliage, spraying was discontinued on these positions. Using the three movable lances only, the quantity of spray applied per 1000 yd. of dike or hedgeside was about 90 gal. This is sufficient for once round a 12-acre field having approximately equal sides. The boom attachment alone delivered spray at a similar rate. When both were used at the same time, about 180 gal./1000 yd. were applied.

Carrot flies are most active in warm dull weather with little wind. Under such conditions the poison bait appears to give the best kill. Spraying gives less satisfactory results when there is a strong wind or when the temperature is below 55° F. as under both

conditions the activity of the flies is low. Dilution of the spray should be avoided by not spraying wet foliage, nor shortly before dew or rain may be expected. When rain falls within an hour of treatment the field should be resprayed at the earliest opportunity.

In the field the persistence of the molasses spray depends chiefly upon weather conditions. It is rapidly washed off by rain, but in dry weather the spray deposit can be seen on the foliage several days after treatment, and probably retains its killing powers. Carrot foliage collected 3 days after treatment gave a 100% kill of flies enclosed with it. With untreated foliage there was a very low mortality. Both lots of foliage were moistened to simulate the effect of dew. Flies collected from fields one day after treatment showed a markedly higher death rate than those taken from untreated carrot fields.

Observations have shown that where conditions remain favourable for insect activity and for spray action over a period of at least 3 hr. after the actual spraying, then a good kill can be expected. In these experiments the interval between successive treatments was either 3 or 4 days except where adverse weather made this impracticable. The 3-day interval was used where high fly populations were encountered, during the peak period of emergence.

Experiments on small-scale control of the second generation, 1942

Creosote as a repellent

The repellent action of creosote to the flies of the second generation was tested at Cambridge. Thick string erected midway between each carrot row and about 2 in. above the soil surface, was well soaked with creosote (applied with a brush) in late July and again in mid-August. Of five plots, each plot consisting of six rows 1 ft. apart and 6 yd. long, three were treated. Two rows of swedes separated neighbouring plots. The carrots were sown in early May, and on 13 Oct. samples of 100 carrots were taken at random from each of the plots. The average attack on the treated and untreated plots is shown in Table 7.

Table 7. *Control of carrot fly by creosote string*, 1942. *Samples taken* 13 *Oct.*

Treatment	Clean %	Unsaleable %	Total mines per 100 carrots	Total larvae in 100 carrots
Treated (av. of 3 plots)	57·3	0·3	70	18·3
Untreated (av. of 2 plots)	29	12·5	181	94

The plots were resampled on 11 Nov., and the average percentages of clean and unsaleable carrots on the treated plots were 30 and 11·3% as compared with 7·5 and 54% respectively for the untreated plots. Similar beneficial results have recently been obtained with creosoted twine in the United States (Hanson & Webster, 1941). This treatment, which is suitable for gardens and allotments, would most probably have been still more effective had the string been re-creosoted at fortnightly intervals from the end of July onwards.

Grass cuttings and 4% calomel dust

Grass cuttings, with and without 4% calomel dust, gave some measure of control of a *moderate* infestation in experiments carried out during 1941. In a similar experiment in 1942, a severe attack was experienced, and on this occasion no reduction in injury resulted from either of the treatments.

Further observations on the biology of the carrot fly

Investigations at Benson's Farm, Chatteris, emphasized the importance of shelter in the distribution of carrot fly under varying conditions of temperature and light intensity. Previous work had shown that the flies seek shelter in rain and high wind.

On 4–6 June sweeping was done at 30 yd. intervals across a 15-acre field of early carrots, and on an adjoining dikeside, hedge and rickyard. The hedge ran in a north-east to south-west direction parallel to and adjoining the dikeside and separated the latter from the rickyard. Nettles covered part of the rickyard up to the hedge and were also present in the hedge and on both sides of the dike. On all 3 days the weather was very hot and sunny with a light north-east wind. The maximum shade temperature recorded was 86° F. at 2 p.m. on 4 June. (G.M.T. is used throughout.)

There was a high fly population in the locality, on one occasion 375 flies were taken from the hedgeside with 100 sweeps of the net. At 6.30 a.m. sweeping showed that large numbers of flies were present on the nettles in the rickyard, on both sides of the hedge and on the dikesides. They were also common on the adjoining headland, but their numbers decreased towards midfield where only a few flies could be found.

At 8 a.m. the number on the carrots had considerably decreased, and by 10 a.m. very few flies could be taken anywhere in the field. This distribution was maintained until about 5 p.m. when the flies again became common on the field and remained there in numbers until about 8 p.m. In the late evening and at 3.30 a.m. few flies could be taken in the field.

The number of flies in the rickyard and on the south-east side of the hedge greatly decreased during the morning as these positions came into the full sun. Over this period they increased on the north-west side of the hedge and in the dike which was then in full shadow. In the late afternoon when the north-

west side of the hedge and the dike itself were in the sun, the greatest number of flies were then to be found in the shade on the opposite side of the hedge.

From this it appears that high temperatures and intense sunlight cause the flies to migrate to shelter, in the manner of an avoidance reaction. Carrot vegetation apparently does not provide sufficient shelter to eliminate the stimuli responsible for this migration and the flies leave the field and remain in the shadow until the heat and light intensity have dropped below a certain level. This is evidenced by the spread of the flies over the field towards evening. A movement of carrot fly into the field from nearby shelter was also observed by Baker *et al.* (1942) in Lincolnshire. This commenced about 4 p.m. and continued into the evening, and is considered by these investigators to be the normal habit of flies during weather favourable to movement. The writers, however, found this rhythm to be associated with heat wave conditions and not to occur in dull warm weather when flies have many times been swept in numbers from carrot crops throughout the day. Under all weather conditions, however, greater numbers have been taken by sweeping from the favoured shelter around fields than from the carrot crop.

The importance of shelter in relation to the degree of attack was emphasized by the sampling results obtained from a 43-acre field of maincrop carrots at Swaffham. This field was bounded on two sides by a hedge, and a hedge with a spinney, and on the other two by trimmed grassy banks. The two pairs of headlands adjoining these and the midfield region were sampled separately and the data obtained are given below:

Position	Carrots clean %	Carrots unsaleable %	Total mines per 100 carrots	Total larvae per 100 carrots
Two headlands near shelter (hedges)	34	8	220	108
Two headlands, little shelter	92	0	12	2
Midfield	98	0	2	0

Attack on seedling carrots

Field observations showed that carrot seedlings are subject to attack at any time *after* the cotyledon stage. However, observations made on experimental sowings at Mepal, where carrot flies were very abundant, showed that it is possible for carrots to be attacked while still in the cotyledon stage. Counts made in June showed that the number of eggs present increased with the size of the carrot foliage, confirming previous observations (Petherbridge *et al.* 1942).

On seedling carrots the young larva first attacks a lateral root or the tap root towards its extremity, and tunnels along it. When the root attains a sufficient diameter, mines are made passing up the tap root to the hypocotyl region, and occasionally as far as the base of the cotyledons. The root, now almost a hollow cylinder, collapses, and the plant shrivels. A small seedling does not appear to provide sufficient food for one larva, and migration occurs, the larva eating through the side and tap roots of neighbouring seedlings and destroying them. A 6 in. strip of a row of closely sown seedling carrots was completely destroyed in this way.

The ability to attack seedling carrots appears to be an important link in the life cycle of the species. On fields growing only maincrop carrots, the first generation of flies is able to reproduce by attacking the seedling stage of the crop in late June and July. Although this attack is generally slight, it gives rise in August and September to the very important and harmful second generation of flies.

Distribution of pupae in the soil

The vertical and horizontal distribution of pupae in the soil adjacent to maincrop carrots was determined on 30 Mar. 1942, at Cambridge. Eight 1 ft. lengths of evenly spaced rows of carrots were used, and the carrots in each carefully lifted. For the vertical distribution a block of soil 12 × 4 in. on either side of the row was removed in layers down to a depth of 8 in., and the pupae removed by washing the soil through sieves. Four replicated sections were taken, and the results obtained are given in Table 8.

TABLE 8. *Vertical distribution of pupae in soil*

Depth of layer examined (in.)	Total pupae in 4 replicate samples	%
0–1	29	22·3
1–2	92	70·8
2–4	5	3·8
4–6	4	3·1
6–8	0	0

TABLE 9. *Horizontal distribution of pupae in soil*

Layer of soil examined (in.)	Total pupae in 4 replicate samples	%
0–1 on either side of mid-row line	47	23·7
1–2 ,, ,,	94	47·5
2–3 ,, ,,	42	21·2
3–4 ,, ,,	15	7·6
4–5 ,, ,,	0	0

For the horizontal distribution four replicate sectionings were made, the soil in each being first removed in a 2 in. strip beneath the carrot row to a depth of 8 in. One-inch strips of soil on either side of this channel to a depth of 8 in. were then removed

in succession to a distance of 5 in. on either side of the mid-line of the row. The pupae counts for each pair of strips are shown in Table 9. The pupae are closely congregated around the carrots, the majority being within 3 in. of the roots and in the top 2 in. of the soil.

Destruction of pupae by soil insecticides

A number of insecticides were tested for the destruction of the over-wintering generation in the soil. These were applied in early April when nearly all the individuals were in the pupal stage. Experiments were carried out at Mepal, and at Cambridge, on clamp sites and on unlifted carrots where previous sampling had shown high pupal populations to exist. As unit areas for treatment, $1 \times 1\frac{1}{2}$ yd. plots were used. The carrots on the plots were lifted just prior to treatment, except where otherwise stated.

On maincrop carrots at Cambridge the treatments shown in Table 10 were carried out. The creosote/sawdust material was made by thoroughly mixing equal weights of each ingredient. A spray gun was used to apply the liquid creosote, a commercial preparation being used in each case. After the treatments, muslin cages were erected in the centre of each plot (covering one-third of its area) to collect the flies as they emerged.

TABLE 10. *Destruction of carrot fly pupae by soil insecticides*

Treatment	Rate per sq. yd.	Notes on treatment	Emergence of flies %
1. Whizzed naphthalene	2·2 oz.	Lightly forked in	17·2
2. ,,	4·4 oz.	,,	10·3
3. Cyanamide	2·2 oz.	,,	138·0
4. ,,	4·4 oz.	,,	110·0
5. Creosote/sawdust	1 lb.	,,	5·8
6. ,,	2 lb.	,,	0·0
7. Creosote (liquid)	½ pint	,,	4·6
8. ,,	½ pint	Lightly raked in	15·0
9. Control I	—	Soil lightly forked over	100·0
10. Carrots lifted Oct. 1941	—	—	4·6

Two plots each of treatments 1–8. Three plots each of treatments 9–10.

The effectiveness of the various treatments was measured by comparing the total number of flies emerging on the treated plots with those emerging on the three control plots (treatment 9 in Table 10). A complete control was given by the creosote/sawdust mixture applied at the rate of 2 lb./sq. yd. Whizzed naphthalene was partially effective, but calcium cyanamide gave no control. Although the plots were distributed at random and well spaced out, a comparatively low emergence was obtained from two of the control plots. Hence it would appear that the maggot attack on the carrots, and the consequent pupal distribution, was not homogeneous, and small differences between treatments must not be taken as significant.

On a clamp site at Mepal, the estimated pupal population was of the order of 5 million/acre: here a lesser degree of control was obtained. As estimated in the previous experiment, the control given by creosote/sawdust mixture at 2 lb./sq. yd. and naphthalene at 4·4 oz./sq. yd. was 86 and 41 % respectively. This is equivalent to an emergence of 14 and 59 % respectively of the emergence in the control plot. At lower rates both gave less control. The effect on plant growth of the insecticides used was tested by sowing white mustard seed on the plots after the period of emergence had ended. At Cambridge the seed was sown in early July and germination and subsequent plant growth were normal on the naphthalene, cyanamide and control plots. On all plots treated with creosote, germination was poor and the seedlings were malformed and later died. At Mepal the seed was not sown until early September, and in this case germination and later growth on all plots, including those treated with creosote, were normal.

From this it would appear that a period of some 6 months is necessary for the phytocidal action of creosote to disappear from the soil. Naphthalene disappears more rapidly, but it appears to be less effective and is more expensive than commercial creosote.

Deterioration of unlifted and clamped carrots

The progress of deterioration due to maggot attack in unlifted and clamped carrots was again followed, and the results obtained in all cases confirmed the results of the previous year (Petherbridge *et al.* 1942). With unlifted carrots deterioration is most rapid from mid-October until mid-December, deterioration being measured most accurately by the number of mines. After December the mines do not increase greatly in number or extent, but deterioration measured by the increase in unsaleable carrots continues; the carrot tissue adjoining the mines becomes blackish, and decay of the whole root may follow. This process is accelerated by frost.

Carrots clamped in October and November deteriorate much less than carrots left in the soil. The mines increase but little in extent, and rotting in the region of the damaged tissue is much less evident; this is probably because the carrots remain comparatively dry in the clamp. Carrots which are found in the autumn to be heavily damaged should not, however, be clamped, but should be lifted immediately and disposed of. In no case should they be ploughed in (a common practice) as this will greatly increase the fly population of that area in the following year.

Comparisons were made of spring emergences from plots lifted in autumn and those unlifted. On maincrop carrots at Cambridge flies emerged from unlifted plots at the rate of 800,000/acre, and from

October-lifted plots at the rate of only 24,000/acre. Also the later in the autumn and winter that carrots are lifted, the larger the population of individuals (as pupae and larvae) which will be left behind in the soil.

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Materials for a study in animal competition

Part III. The seasonal rhythm of *Psychoda alternata* Say and an effect of intraspecific competition

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(With 1 Text-figure)

The incidence of the sewage fly, *Psychoda alternata* Say, breeding almost free from other insect competitors in a bacteria bed at Huddersfield is examined. Its general seasonal trend is marked by periods of peak output explained on the theory that there are two successions of generations running persistently and alternating with one another. Such behaviour has previously been demonstrated for another sewage fly, *Spaniotoma minima*, and has been shown to be instituted by irregular temperatures in autumn and spring. In *Psychoda alternata* a special type of intraspecific competition is set up in the warmer months when the cycles are rapid, for the larvae of one succession must start life in a bed just depleted of food by the other. Therefore large and small outbursts of the fly tend to alternate. The discharges of solids from the bed have a detailed periodicity corresponding to the alternations in the successions of the fly.

Psychoda alternata Say is the most widespread of the bacteria bed flies and the one most tolerant of strong sewage so that it persists and thrives almost unhampered by interspecific competition in certain beds such as those of Huddersfield where chemical wastes characterize the sewage. The behaviour of the insect was studied at Knostrop (Leeds) where interspecific competition is intense (Lloyd *et al.* 1940; Lloyd, 1937, 1943), at Barnsley where the successful species are fewer than at Knostrop (Lloyd, 1937), and in the restricted conditions at Huddersfield (Golightly, 1940; Reynoldson, 1941). At all these places its seasonal incidence, assessed on a monthly basis, is characterized by zenith periods in early summer and in autumn respectively with an intervening depression. The extension of its period of abundance into the colder months at both ends of the year is most pronounced at Huddersfield, mainly as a result of species restriction and partly because the beds are slightly warmer than those of Knostrop or Barnsley. Its seasonal incidence has not been examined in detail hitherto, and an analysis of this is now given, as the opportunity for such an insect study in the field rarely occurs and also because it throws light on an important matter in sewage disposal, the seemingly erratic fluctuations

in the amount of solids discharged in the final effluent.

P. alternata is essentially a summer insect of crepuscular habits, creeping and clustering into crevices in strong light. It normally takes to flight on emergence but need not do so as it can mate and complete the cycle in total darkness. Thus it continues to breed at a reduced rate in the depths of the beds through the winter months since there is no diapause in the cycle. Progress becomes very slow and mortality proportionately high when temperature falls near the thresholds of egg development and larval growth (Lloyd *et al.* 1940). In the cold months it does not appear at all at the surface of the beds at Knostrop or Barnsley but is to be trapped in most weeks at Huddersfield though the numbers caught in midwinter are very low.

The material used in this study was from trappings made through 31 months at Huddersfield on the secondary bed of a double filtration plant the biology of which was being studied by Reynoldson (1941, 1943). The insects trapped by routine method (Lloyd *et al.* 1940) were sent to the author for examination and Reynoldson (1941) recorded the general composition of the fauna. *P. alternata* comprises 98% of the total insect catch. It is assessed by rough estimates in a dimly lighted tray on the bed, from a dark tin, and from a glass jar collecting the insects that take flight from the tray which covers 1 sq. ft. of bed. Reynoldson (1941) showed that the three methods at Huddersfield give virtually parallel results so only the jar record is considered in this account. The jar was changed weekly and as the females mature and oviposit 2–3 days after emergence in warm weather many no doubt in this interval pass back on to the bed while a week under summer conditions is more than the natural span of imaginal life. The numbers recorded should therefore be regarded as a weekly sampling rather than as a week's accumulation of emergences. The record of the catches with the mean weekly bed temperatures are given in Table 1. The analysis was made from the daily means but the calculations can be roughly checked from the weekly mean temperatures. The tracing of the life cycles was done by the following constants (Lloyd, 1937):

	Threshold ° F.	Day degrees required ° F.
Maturation of female	35·5	83
Incubation of egg	42·5	36
Larval growth	42·0	439
Pupation	46·0	56

The following account can be followed in Fig. 1 (*a*)–(*c*). Taking the first peak appearance of the fly at 8 Nov. 1939, as a starting-point, the next generation of fly is due on 10 Jan.; from 11 Jan., the next is due on 19 Apr. and so on. In this way twenty completed cycles are possible up to 27 May 1942, a week before the record ends. These are indicated by vertical numbered lines. It is evident that there is a corresponding rhythm in the curve of fly incidence though not an exact fit. Two or sometimes three peak periods roughly correspond to the cycle except that in the height of summer on occasions there is only one broad-based peak. This demonstrates that there are two successions of generations running as has already been shown for another sewage fly, *Spaniotoma minima* (Lloyd, 1941). The phenomenon is due to irregularities in the rising spring temperature which tend to form the peaks and to irregularities in the autumn cooling which tend to split them. It has been shown that an originally uniformly distributed population of an insect with rapid cycles proceeding without diapause would in a few years time tend to assume this form of seasonal rhythm. Broods established on succeeding days in cold weather tend to cluster to a shorter interval with rising temperature and so the cores of peaks are formed and these cores are very persistent, being overlaid by the general seasonal level of emergences. If one of the sudden characteristic autumn temperature falls happens at a period of peak emergence, the peak splits and the daughter peaks may, or may not, rejoin in spring depending on the chance whether at the end of the cycle a compensating rise of temperature is encountered. The manner in which the established peaks expand and tend to split and then again contract and sometimes rejoin is shown for the three winters of this record of *P. alternata* by the calculated bars in the upper part of the diagram. The successions which are labelled *A* and *B* are traced as follows. Generally a tracing begins from the trapping day just following the calculated emergence but as the record becomes confused when the peaks run together, some of the bridging has been done by assessing temperature backwards and a gap of more than a week may thus be formed (see p. 362).

It was said above that 20 cycles should be possible in the 31 months, but succession *A* achieves 19 and *B*, starting 14 days later, covers 18½ cycles. The diagram shows that the missing cycle is lost fairly steadily as the vertical lines gain on succession *A*. The cycles of the fly in the bed are thus somewhat slower than the formula demands. The food of the larvae is mainly, if not entirely, the growth on the medium which in this case owes its bulk mainly to the fungus *Oospora*. Reynoldson (1943) showed that in this bed the growth is thick only in spring, that it is very much depleted by the grazing larvae through summer and autumn when its increment should otherwise be greatest, and that while the larvae are relatively inactive through winter, cold prevents much increase. Therefore, there is direct evidence of periodic depletion of food and this probably to some extent slows up larval progress as opposed to laboratory experience.

Several times in the record there is evidence of over-population and consequent depression through

excessive mortality. This is shown by the regular alternation of high and low peaks of emergence from 1 May to 2 Oct. 1940 and again from 25 June to 8 Oct. 1941. The low peak following the latter date might be partly depressed also from this cause, but there is also a splitting of the succession at this point, temperature suddenly falling. Such alternation is doubtless due to periodic intensification in intraspecific competition which the alternation of the two successions must induce. Thus the eggs of succession *A* from 10 Apr. 1940 are due to hatch on 17 Apr. when the bed carries the larvae about half grown of succession *B* due to emerge about 1 May. Succession *A* shows a partial collapse on emergence on

TABLE 1. *Record of* Psychoda alternata *trapped and of mean bed temperatures. Relative weight of solids in the bed effluent (parts per* 100,000) *shown from June* 1941. *All records weekly*

Week ending	Fly trapped	Bed temp. ° F.	Week ending	Fly trapped	Bed temp. ° F.	Week ending	Fly trapped	Bed temp. ° F.	Solids in effluent
1939 1 Nov.	145	51	1940 21 Aug.	180	63	1941 11 June	541	59	0·60
8	293	58	28	11	63	18	260	62	1·40
15	163	56	4 Sept.	184	66	25	641	67	0·60
22	344	56	11	98	63	2 July	130	67	0·45
29	15	52	18	160	59·5	9	320	67	1·00
6 Dec.	80	49·5	25	98	59	16	490	69	2·10
13	3	50	2 Oct.	550	59	23	256	65	1·20
20	3	51	9	160	59	30	234	66·5	0·80
27	No record	51	16	650	57	6 Aug.	150	64	0·55
1940 3 Jan.	3	49	23	420	61	13	122	61	0·60
10	29	52	30	145	55	20	265	61	0·80
17	3	49	6 Nov.	21	54	27	73	62·5	1·70
24	0	46·5	14	17	50	3 Sept.	353	63	0·40
31	0	45	21	33	48	10	65	66	0·40
7 Feb.	0	47	28	71	50	17	141	64	3·00
14	1	43	4 Dec.	93	51	24	85	64·5	2·50
21	1	46	11	37	46	1 Oct.	36	65	1·60
28	14	46	18	70	48·5	8	502	64·5	5·00
6 Mar.	28	46	25	6	49	15	151	58·5	1·30
13	10	50	1941 1 Jan.	1	48	22	200	56·5	1·30
20	1	48	8	39	42	29	48	52	1·40
27	5	50	15	38	47	5 Nov.	415	49	2·20
3 Apr.	157	51	22	72	47	12	190	53·5	2·70
10	233	53	29	0	50	19	74	54·5	1·80
17	190	54	5 Feb.	3	50·5	26	49	53	1·50
24	13	52	12	2	51	3 Dec.	30	53·5	1·50
1 May	510	58	19	0	51	10	81	52	1·80
8	198	61	26	0	50	17	No record	50	1·30
15	249	61	5 Mar.	1	52·5	24	10	50·5	No record
22	312	63	12	4	51·5	31	5	49	1·70
29	216	65	19	125	54	1942 7 Jan.	22	48	0·70
5 June	350	66	26	21	56	14	8	49	0·30
12	800	69	2 Apr.	0	52	21	No record	48·5	No record
19	450	67	9	0	52	28	0	48·5	0·15
26	250	65	16	2	53·5	4 Feb.	0	48·5	0·35
3 July	275	65·5	23	212	56	11	0	48·5	2·00
10	210	64·5	30	300	52·5	18	0	49	1·30
17	270	66·5	7 May	126	56·5	25	1	47	0·20
24	250	63·5	14	300	54·5	4 Mar.	3	45	6·80
31	900	65·5	21	424	57	11	1	48	0·55
7 Aug.	320	69	28	300	58	18	5	51	1·60
14	240	63	4 June	320	60·5	25	5	52·5	5·30
						1 Apr.	4	51·5	1·50
						8	0	52	0·90
						15	113	56·5	9·50
						22	195	54·5	2·10
						29	34	57·5	2·90
						6 May	546	55·5	6·30
						13	470	60·5	1·70
						20	501	60·5	1·40
						27	167	58	2·00
						3 June	473	59	0·75

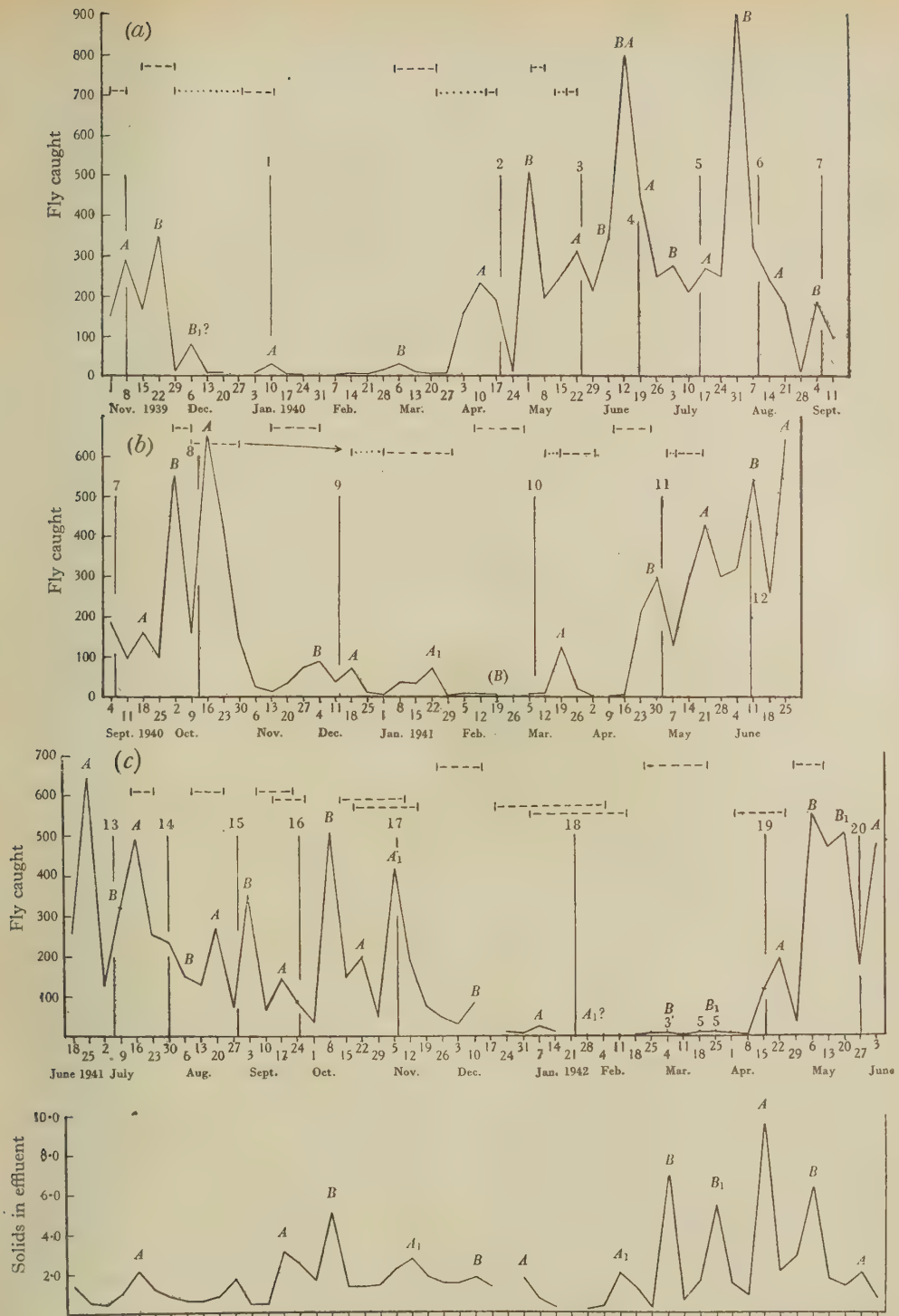
(a)
Fly caught
Nov. 1939 Dec. Jan. 1940 Feb. Mar. Apr. May June July Aug. Sept.
(b)
Fly caught
Sept. 1940 Oct. Nov. Dec. Jan. 1941 Feb. Mar. Apr. May June
(c)
Fly caught
June 1941 July Aug. Sept. Oct. Nov. Dec. Jan. 1942 Feb. Mar. Apr. May June
Solids in effluent

Fig. 1. The incidence of *Psychoda alternata* as indicated by trapping through 31 months. The numbered upright bars show the intervals between rapidly succeeding generations and the lettering of the peaks of fly indicate that two successions of generations are running. The horizontal bars show how the peaks expand and retract under the influence of temperature change. The bottom of Fig. 1 (*c*) shows the fluctuations of solids passing with the bed effluent. (The three sections of the diagram have some overlap.)

Generation no.		Succession *A*		Succession *B*	
		Calculated cycle	Actual peak	Calculated cycle	Actual peak
1	1939	8. xi–10. i	10. i	15. xi–5. iii 22. xi–19. iii	6. iii
				B_1 6. xii–23. iii (B_1 is lost, probably joins *A*)	
2	1940	10. i–17. iv	3–17. iv	6. iii–4. v	1 v.
3		3. iv–15. v 17. iv–22. v	22. v	1. v–30. v	in complex
4		22. v–15. vi	in complex (peak is at 12. v with contributions from both *A* and *B*)		
				5. vi–29. vi	3. vii low
5		19. vi–15. vii	17. vii low (both successions thus appear to emerge as low peaks from the complex)		
				3–29. vii	31. vii
6		17. vii–11. viii	in complex (this region is doubtful but the two successions emerge when about due)	31. vii–26. viii	4. ix low
7		14. viii–10. ix	18. ix low	4. ix–7. x	2. x
8		18. ix–23. x	16. x	2. x–19. xi 9. x–3. xii (spread with falling temperature)	14. xi–4. xii
9		16. x–21. xii 23. x–25. i (split with falling temperature)	18. xii *A* 22. i A_1	14. xi–14. ii 4. xii–3. iii	(depressed by cold, mean air temperature from 14. ii to 12. iii was 37° F.)
10		*A* 18. xii–12. iii		12. ii–12. iv	
	1941	A_1 22. i–25. iii (temperature rise effects reunion)	19. iii	5. iii–28. iv	23–30. iv
11		19. iii–8. v	14–21. v	30. iv–8. vi	11. vi
12		21. v–22. vi	25. vi	11. vi–6. vii	in complex
13		25. vi–18. vii	16. vii in complex	9. vii–2. viii	in complex
14		16. vii–11. viii	20. viii	9. viii–4. ix	3. ix
15		20. viii–16. ix	17. ix	3–29. ix	8. x
16		17. ix–16. x 24. ix–31. x (split through falling temperature)	22. x *A* 5. xi A_1	8. x–28. xi 15. x–10. xii (missed record on 17. xii)	10. xii
17		*A* 22. x–23. xii		28. xi–26. ii *B*	4. iii
		A_1 5. xi–19. i (missed record on 21. i: depressed by intense atmospheric cold)	7. i	10. xii–23. iii *B* (evidence slight but confirmed below)	18–25. iii
18	1942	7. i–5. iv		*B* 4. iii–2. v	
		11. ii–23. iv (*A* and A_1 would rejoin here)	22. iv	B_1 18. iii–7. v 25. iii–14. v	6–20. v
19		22. iv–30. v	3. vi	end of record	

22 May. Owing to this depopulation the next generation of *B* is less interfered with, the eggs being placed in a less densely crowded bed. The cycles being very rapid a complex peak representing an abundant emergence follows. This mass development seems again to deplete the bed of food and great mortality results in the next cycles, both successions being depressed on 3 and 17 July respectively. Food conditions again improve in consequence and *B*, being ahead of *A*, is able to produce a thriving brood at the apex of the peak on 31 July with *A* making no impression on the decline at 14 Aug. With food again depleted *B* and *A* both show depression on 4 and 18 Sept. respectively with recovery in the next cycles which are now slowing down. In the spring of 1941, in a bed presumably now fully loaded with growth, the population increases through five steadily ascending peaks to a zenith on 25 June. The record shows that from this point the sequence of over-population-depleted bed-population decline-food recovery-population recovery begins again. In the following spring there is steady increment in a bed known to be well loaded (Reynoldson, 1943). This alternation of high and low periods of fly output seems to be confined to the warmer months when the cycle of the insect is less than 30 days. The successive loads of larvae are then superimposed one on the other with rapidity and potential family size

is at a maximum (Lloyd *et al.* 1940). In winter not only are the peak loads of larvae more widely spaced but the egg and young larval mortality at low temperatures would counter excessive populations.

It was hoped that a record of fly size would throw light on the details of intraspecific competition and size was determined by the wing length of the females in each weekly batch through 2 years. A difficulty expected is that size is very responsive to temperature change during late larval growth, but it was thought that this could be allowed for by using a scale of size-temperature relationship (Golightly, 1940). Through the colder months the flies were well up to average size and almost uniformly below normal through the seasons of intenser competition. The most notable collapse was at 25 June–2 July 1941; on the former date, wing length 2·16 mm. was expected and 1·79 realized and on the latter, temperature being higher, 2·02 mm. was expected and 1·70 realized. These flies are minute especially when contrasted with winter flies which have a wing length of about 2·5–2·6 mm. However, in general, there was no conformity between size and population pressure as indicated by emerging fly. In this insect intraspecific competition influences numbers far more distinctively than individual size. This may be because when a brood relatively fails it generally does so near its foundation through juvenile mortality. Lloyd (1943), discussing interspecific competition in the Knostrop beds, produced evidence that where the numbers of one species decline under the influence of another, this influence is mainly exerted at the establishment of brood rather than later in the cycles.

Solids in the bed effluent comprise fragments of film loosened from the medium, the dejecta of grazing organisms, cast insect skins, dead insects and so on. Reynoldson (1943) provided a weekly record of these solids from 11 June 1941 and his figures are reproduced in Table 1 and plotted below the curve of fly incidence in Fig. 1 (*c*). He noted the heavy discharges of solids during spring, vernal slough, when the accumulated growth of winter is being broken down by the rapidly increasing activity of the grazing larvae, but also that spasmodic fluctuations in solids occur throughout the year. He has not attempted to deal with these because, as he has stated to the author, the fluctuations are pronounced in winter when the scouring of the few larvae he encountered in excavations of the bed must be negligible. Also the numbers of larvae trapped by bait in winter are low. It is submitted, however, that many of these spasmodic increases in solids are to be explained on the theory of the succession of alternating generations which indicates when peak periods of activity are due. In winter, atmospheric cold prevents the flies from showing at the surface in distinctive numbers while excavation of the bed in search for larvae can give only local and rather shallow findings. The trapping of larvae by bait in winter is not likely to give results equivalent to those of warmer weather for two reasons. First, larvae are less active during cold weather and so less likely to discover a bait and, secondly, as the intensity of the competition is reduced, food is sufficient for larval needs and the counter-attraction of a bait so much the less. It is certain, however, that there must be many larvae somewhere in the bed to give rise to the large emergence of fly in spring.

Reference to Fig. 1 (*c*) shows 13 peak weeks of discharged solids of which 12, appropriately lettered, correspond to peak intervals of fly output or to times when peak periods are due but no fly, or only trifling numbers, appear in the traps. Thus on 11 Feb. the peak A_1 is about due but no fly could be expected because the weather is very cold with the country snowbound. There is a discharge of solids and about this time there is laid down the brood of fly which gives rise to the peak with apex at 22 Apr. The exact bridging is as follows: offspring from 8 Nov. is due on 24 Jan., offspring from 25 Jan. is due on 16 Apr. Similarly the heavy discharges of solids on 4 and 25 Mar. are accompanied by small evidence of fly but at this time emergence of succession *B* from brood laid down about 10 Dec. is due and must have been considerable, for from brood laid down in March the very high emergences of 6–20 May result.

Thus, it is shown that the discharges of solids are not erratic events but have a certain rhythm which is dependent on the rhythm set up in the incidence of larvae by the alternating successions of generations of the fly. The timing of the rhythm is dependent partly on current temperature and partly on more distant temperature effects. This is perhaps the best evidence yet produced from the field of the very intimate relationship between the behaviour of the bed and the activity of grazing organisms. It has been possible to demonstrate it because of the restricted fauna of the Huddersfield beds. In beds supporting a variety of successful organisms, worms and insects, periods of special grazing activity would not stand out as rather isolated events and discharges of solids could therefore be tied with less precision to particular species and peaks of fly output.

Dr Reynoldson has kindly placed all his data at my disposal for the framing of this account and has discussed the draft of it submitted to him.

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The stem and bulb eelworm, *Anguillulina dipsaci* (Kühn, 1858): the importance of collating evidence on the behaviour of biologic strains

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(With Plates 7 and 8 and 1 Text-figure)

The results of experiments on the infection of many different plants with seven biologic strains of known history of the nematode, *Anguillulina dipsaci* (Kühn), are given, together with the results obtained by other investigators. The technique by which the nematode was induced to infect other host plants is described in detail. The biologic strain theory in relation to *A. dipsaci* is discussed, together with the merits of the trinomial system of nomenclature in connexion with unspecialized races. The factors concerned with nematode outbreaks receive consideration. The relationship that exists between flower colour in herbaceous phloxes and the degree of infection by *A. dipsaci* is presented.

INTRODUCTION

The objects of this paper are to place on record the results of some recent investigations on the behaviour of certain biologic strains of *Anguillulina dipsaci*; and to indicate the relationship that exists between colour in herbaceous phlox flowers and eelworm infection.

The existence of biologic strains or races of this nematode has long been recognized, and the subject has been reviewed by a number of investigators, notably Goodey (1931, 1933, 1936) and Hodson (1926*a*, 1931) in this country, and by Steiner (1925) and others in the U.S.A.

According to Steiner, it appears that a plant parasitic nematode prefers the host species or even the host variety in which its parents lived. The attractiveness of certain hosts increases with the number of generations the eelworm population is associated with them. This specialization may eventually reach such a high degree that finally even other plants with the closest taxonomical, physiological and chemical relationship to the primary host are either not attacked or only feebly or temporarily invaded.

The behaviour of certain strains confronted with a single plant species, i.e. as in a 'starvation' test, shows that the eelworm population of a race hitherto considered specialized will often attack a plant which would be ignored if a more attractive one was present. It is clear from this fact that there is value in submitting biologic strains of plant-parasitic nematodes to both 'starvation' and 'host selection' tests.

The theory concerning the presence of biologic races is, according to Goodey (1931), 'that they represent adaptations, such that, the longer a parasite attacks a particular host the higher the degree of specialization to it becomes and the less it is able to attack other plants'.

WISLEY INVESTIGATIONS

Investigations carried out in the Royal Horticultural Society's Gardens at Wisley, Surrey, during the past few years aimed at determining the host range of a few of the many biologic strains of this nematode, namely, the hyacinth, iris, narcissus, *Oenothera*, onion, phlox and potato races. Data were accumu-

lated on the ability of known races to infect other plants, and considerable attention was paid to employing a strain associated for a number of years with a particular host (Tables 1, 2 and 3).

A number of workers, both in this country (Goodey, 1923 *et sqq.*; Edwards, 1937, 1940; Hodson, 1926*a*, 1931; Johnson, 1936*a*, 1939; and others), on the Continent (Bos, 1888–1892; Quanjer, 1927); in the United States (Godfrey, 1924; Kreis, 1937; Steiner, 1925), and elsewhere, tested the ability of certain known races of *A. dipsaci* to infect other host plants. It was clearly shown that many strains are unable indiscriminately to transfer from one host to another. Some strains are highly specialized and are restricted to one or two host plants (monophagy), some are less discriminate in their choice of hosts and are capable of infecting a limited number of plants (oligophagy), while others are unspecialized and are capable of infecting and reproducing in several and often dissimilar hosts (polyphagy).

It is known that some, though not all, strains are reciprocally infective. For instance, an onion strain used in the Wisley experiments readily infected narcissus bulbs, while another did not do so even in the absence of any alternative host. Reciprocal infection is not a constant factor and the behaviour of any strain is dependent upon its ancestral history. Conflicting results obtained by workers in host-transference experiments may be due to the varied ancestral history of the strains used.

The practical outcome of such investigations is, however, suspicion of any race of this nematode that has at one time been naturally or artificially transferred successfully from one host to another. For instance, growing nerine bulbs in ground known to contain the narcissus strain may result in some infection in a succeeding crop of narcissus (Table 1). The fact that even one investigator has observed or set up infection in nerine with a narcissus strain is sufficient evidence upon which to advise the grower to avoid following this one crop with the other.

Some strains when injected into other hosts will live for a time and even produce local cellular disturbance (Pl. 7, figs. 1 and 2), but do not persist and do not appear capable of breeding in their new environment (Goodey, 1941*a*). Any plant that has been induced to become even slightly infected by a particular strain may become highly infected after a time when the eelworm has been able to adapt itself to its new host.

A plant temporarily infected is not to be generally considered as a reservoir host of the strain present in the soil.

Technique. Experimental work on biologic strains of *A. dipsaci* should be restricted to laboratory observations because both in highly cultivated soils, where a succession of different plants have been grown over a number of years, and in freshly broken pasture where there is often a varied flora, one or more biologic races may exist. The investigator is, therefore, uncertain as to what strains are present in the soil. Critical work is necessarily restricted to pot or cylinder experiments and the strain used should be one whose ancestral history is known.

The Wisley experiments were carried out with biologic strains whose immediate ancestral history was known, and covered periods of 5 years (minimum) and 10 years (maximum). The technique employed was as follows:

(1) Seeds were sown in 12 in. pans containing sterilized soil which was infected with a known strain of *A. dipsaci* by watering the soil with a suspension obtained by macerating tissue of the infected primary host. 'Starvation' tests were made by sowing one species of plant in each pan (Pl. 8, fig. 4), while 'host selection' tests were made by sowing two, three or four different species and/or varieties of plants in each pan to permit the strain to exhibit its host preferences (Pl. 8, fig. 5).

(2) Seeds were sown in 12 in. pans in soil infected by incorporating with the sterilized compost diseased material which had been dried and ground.

(3) Bulbs and corms were injected directly with a suspension of nematodes placed in sterilized glass tubes with a finely drawn-out end to allow the eelworms to pass down singly into the plant (Pl. 7, fig. 3).

(4) Plants were infected by the drip method whereby the eelworms dropped down through the finely drawn-out end of glass tubes on to the foliage, bulb, corm, stem and bud axil.

All infected annual plants were kept under observation for 6 months, while bulbs and perennials were allowed to remain for 12–24 months.

The value of 'starvation' tests is limited for they do not invariably indicate the true behaviour of a strain owing to the restricted choice of food provided.

The attractiveness of a number of Polemoniaceous plants to the *Phlox* strain was tested, and (Table 3) only four (*Collomia*, 1 sp., *Gilia*, 3 spp.) were susceptible, and that ten other species of *Gilia* were not attractive in either the 'starvation' or the 'host selection' tests. It is, however, unlikely that none of the other ten species of *Gilia* is a possible host of the *Phlox* strain, and it would be unwise to risk growing any species of *Gilia* in ground known to contain the *Phlox* race.

The results obtained are given in Tables 1, 2 and 3, and the symbols used to signify infection or freedom from infection to biologic races are similar to those adopted by Godfrey & Scott (1935); they are as follows: ++ signify severely, + moderately, and (+) feebly or temporarily infected, while − indicates that no infection was obtained.

TABLE 1

The following biologic strains of *A. dipsaci* were investigated at Wisley with a view to ascertaining the degree of adaptability of each strain to infect other host plants. References are given to the results obtained by other investigators, which in the greater number of cases agree with the results obtained at Wisley.

	+	+	−
(i) *Hyacinth strain.*	Hyacinth →	Hyacinth Onion (Bos, 1888, 1888–92) Scillas (Bos, 1892)	*Galanthus nivalis* (Slogteren, 1920) Narcissus (Slogteren, 1920)
(ii) *Iris strain.*	Iris, bulbous →	Iris, bulbous	Narcissus (Steiner & Buhrer, 1933; Hodson, 1934) Onion (Steiner & Buhrer, 1933)
(iii) *Narcissus strain* (see Table 2 for Wisley experiments).	Narcissus →	*Allium triquetrum* (Goodey, 1929) *A. vineale*, N1, N3 (Hodson, 1931) *Coronopus Ruellii* (Goodey, 1937*b*) *Hypochaeris radicata*, N2, N3 (Hodson, 1931) Narcissus Nerine (Radcliff, *in litt.* 1930) Onion (Ramsbottom, 1919; Newhall & Chitwood, 1940) *Plantago lanceolata*, N3*a*, N4, N6 (Hodson, 1931) Scilla (Ramsbottom, 1918) *Sprekelia formosissima* (Slogteren, 1920) Strawberry (Hodson, 1938)	*A. vineale*, N2, N4, N5, N6 (Hodson, 1931) Broad beans (Ramsbottom, 1919) Buckwheat (Ramsbottom, 1919) Chives (Ramsbottom, 1919) Clover (Ramsbottom, 1919; Hodson, 1926*a*) Gladiolus (Leak, 1927) Hyacinth (Slogteren, 1920, 1923) *Hypochaeris radicata*, N1, N4, N5, N6 (Hodson, 1931) Iris, bulbous (Staniland, 1934, 1936) Lucerne (Ramsbottom, 1919) Nerine (Fox Wilson, 1930) Oats (Ramsbottom, 1919; Hodson, 1926*a*) Peas (Ramsbottom, 1919) Phlox (Steiner & Dodge, 1929) *Plantago lanceolata*, N1, N2, N3*b*, N5 (Hodson, 1931) Potato (Ramsbottom, 1919; Buckhurst, 1927; Fox Wilson, 1927) Rye (Ramsbottom, 1919) Rye grass (Ramsbottom, 1919) Strawberry (Hodson, 1926*b*, 1938) Wheat (Ramsbottom, 1919)
(iv) *Oenothera strain.*	*Oenothera* →	*Oenothera* spp. *Phlox paniculata*	Narcissus Onion
(v) *Onion strain.*	Onion →	Narcissus Onion Parsnip (Goodey, 1937*b*; Walton, 1936) Rye (Bos, 1888–92)	Beetroot (Newhall & Chitwood, 1940) Carrot (Newhall & Chitwood, 1940) Clover (Hodson, 1926 *a*) Leeks Lettuce (Newhall & Chitwood, 1940) Oats (Hodson, 1926*a*) Spinach (Newhall & Chitwood, 1940)
(vi) *Phlox strain* (see Table 3 for Wisley experiments).	Phlox →	Gladiolus (Leak, 1927) Peas (Den Brock & Schenk, 1915) Potato (Den Brock & Schenk, 1915) *Primula chinensis* (*sic*) (Den Brock & Schenk, 1915)	
(vii) *Potato strain.*	Potato →	Beans (Quanjer, 1927) Cabbage (Quanjer, 1927) Mangold (Quanjer, 1927) Peas (Quanjer, 1927) Many other plants (Quanjer, 1927) Potato	Barley (Goodey, 1935) Mangold (Goodey, 1935) Narcissus Oats (Goodey, 1923, 1935; Hodson, 1926 *a*) Onion (Goodey, 1935) Peas (Goodey, 1935) Rye (Goodey, 1935) Turnip (Goodey, 1935) Wheat (Goodey, 1935)

Table 2. *Cross infections with* Narcissus *strain*, 1938–1939

The previous known history of this strain in 1938 was 10 years exclusively in *Narcissus Pseudo-Narcissus*.

++	+	(+)	−
Onion	*Narcissus cyclamineus*	*Allium Ostrowskianum*	*Brodiaea uniflora*
	N. poeticus	*Asphodeline lutea*	*Colchicum autumnale*
	N. Pseudo-Narcissus	*Scilla nonscripta*	*Convallaria majalis*
		Parsnip	*Crocus vernus*
			Erythronium Dens-canis
			Hyacinthus amethysinus
			Iris histrioides major
			I. tingitana
			I. xiphioides -
			I. Xiphium
			Muscari comosum

Seeds of the above-mentioned bulbs were sown in 1937 in sterilized soil in pans, while those of parsnip were sown in March, 1938 and 1939. The pans were watered with a suspension of *A. dipsaci* in March, 1938 and 1939, obtained from heavily infected narcissus bulbs.

Table 3. *Cross infections with* Phlox *strain*, 1924–1937

The previous known history of this strain from 1924 was 6 years exclusively in *Phlox paniculata*.

+ +	−
Oenothera fruticosa Youngii	*Allium Cepa*
Oe. glauca Fraseri	*Anthoxanthum odoratum*
Phlox Drummondii cuspidata	*Campanula Medium*
P. D. grandiflora	*C. persicifolia*
P. D. nana compacta	*Cheiranthus Allionii*
P. paniculata	*Cobaea scandens*
P. suffruticosa	*Delphinium Ajacis*
	Gilia abrotanifolius
+	*G. androsacea*
Collomia coccinea	*G. capitata*
Gilia achilleaefolia	*G. densiflora*
G. coronopifolia	*G. dianthoides*
G. minima coerulea	*G. laciniata*
Oenothera biennis	*G. liniflora*
Oe. fruticosa	*G. micrantha*, 4 vars.
Oe. fruticosa major	*G. nivalis*
Oe. Lamarckiana	*G. tricolor*
Oe. odorata	*Gladiolus hybridus*
Phlox divaricata	*G. primulinus*
	Narcissus Leedsii X
(+)	*N. poeticus*
Dianthus barbatus	*N. poeticus ornatus*
Schizanthus retusus	*N. Pseudo-Narcissus*
S. wisetonensis	*Phlox Arendsii X*
Solidago canadensis	*P. pilosa*
	P. subulata
	Plantago lanceolata
	P. major
	Polemonium caeruleum
	P. humile
	P. occidentale
	Polygonum Persicaria
	Primula rosea
	Taraxacum officinale
	Trifolium incarnatum

Seeds of all the above-mentioned plants were sown in sterilized soil in pans either annually (annuals) or during the year prior to artificial infection (bulbs and perennials). The pans were watered with a suspension of *A. dipsaci* obtained from heavily infected herbaceous phloxes.

Nomenclature of biologic strains

It is unnecessary to discuss in detail the desirability of recognizing the existence of biologic races in nomenclature, but the attention of biologists is drawn to the food-memory hypothesis outlined by Goodey (1931) and to the adaptation hypothesis presented by Ouboter (1930).

The use of a subspecific name, i.e. that of the host, has been adopted by some investigators, namely, Ouboter (1930), Steiner & Scott (1934) and others, who claim to identify and to separate biologic races by one or more minute differences in their morphology and biometry. It is apparent that the binomial system must stand for unspecialized or polyphagous strains, whereas for the race that is highly specialized it is suggested that the trinomial system be adopted.

The validity of the adoption of any such nomenclature must depend upon whether host-avoidance in monophagous races is a constant character, and only long-term research on the behaviour of such strains will provide data upon which to judge the merits or demerits of the system. Moreover, with *A. dipsaci*, the issue may be confused owing to the fact that this nematode exhibits pronounced size differences, e.g. length of tail (*forma brevicaudata*, *media*, and *longicaudata*), and the occurrence of giant races in the same host from different geographical localities as described by Goodey (1941 *b*). Also, it is as yet unknown whether even a highly specialized strain may become less specialized over a period of time and capable of adaptation to other hosts when frequent changes of crop are grown in a particular area where hitherto a highly specialized strain existed.

It is the author's considered opinion that the adoption of the trinomial system is premature.

Practical considerations

The question that concerns the applied biologist is how far our present knowledge allows a definite statement as to crops that should be avoided in eelworm-infected soil. Our knowledge of the be-

haviour of biologic strains is meagre, and it is, therefore, desirable to collate the evidence already available and to present such data in a readily intelligible form for the agricultural and horticultural community.

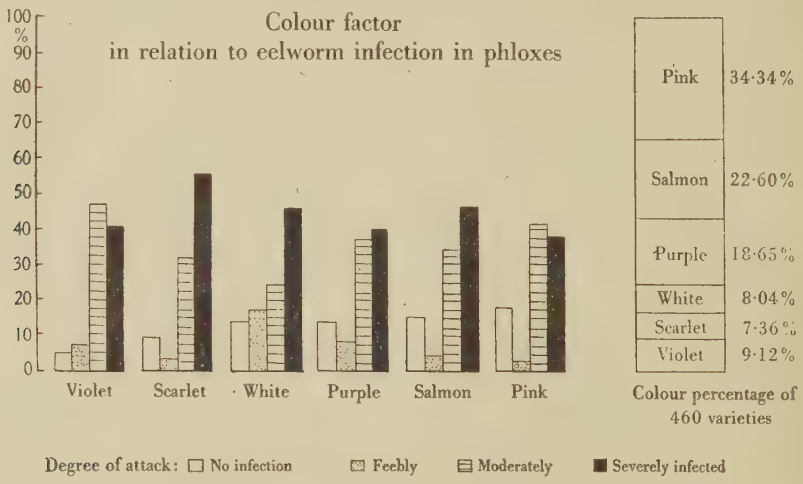
When considering the measures for cleaning-up infected areas, a knowledge of crop history of the land and of the race of eelworm present will enable the grower to avoid infection by withholding susceptible plants known to be attractive to the strain or strains in the area under consideration. Clean cultivation to eradicate weed hosts, and the complete lifting of the crop to avoid the presence of 'volunteer' plants is also essential. The importance of removing all crop remnants is emphasized though the task of clearing large areas of bulbs or tubers of groundkeepers, or of stubble and straw may need considerable effort.

As Steiner (1925) suggests, it is desirable to exclude perennial plants from any crop rotation system in infected fields, in order to avoid the possibility that the nematode race may adapt itself to the perennial host over a number of generations.

Further data are urgently required on the behaviour of specialized and unspecialized races occurring in weeds, and the wild flora of land infected with *A. dipsaci* needs careful examination. Such data may throw some light upon the persistence of the pest in fields where a 4–5 years' crop rotation system is practised. The possibilities of weeds as 'bridging' or 'reservoir' hosts is important, and the work of Goodey, Hodson, Johnson and others should be emulated.

In connexion with the sporadic outbreaks of *A. dipsaci* that occur from time to time on onion crops, data are urgently needed as to the origin of such infestations, especially those that occur in freshly converted pasture and grassland. Several such outbreaks have been confirmed by the writer during recent years in freshly broken land where none of the wild species of *Allium* recorded as hosts of the eelworm is known to exist. It is possible that infection is seed-borne, while the alternative is that there existed in such land a biologic race capable of transferring from some gramineous or other pasture plant to onions.

It has long been known that *A. dipsaci* is transmitted in or on seeds (Kühn, 1858; Godfrey, 1924; Goodey, 1939; Hodson, 1929), while Bos (1888) recorded the presence of this nematode in the flowers of *Allium Cepa*, and found that 3% of the seeds were infected.



Text-fig. 1.

Colour grouping of phloxes in relation to eelworm infection

It has been stated that there may occur some correlation between the colour in herbaceous phlox flowers and the incidence of attack by *A. dipsaci*. For instance, statements have been made (*in litt.* 1934, 1935) that mauve varieties were definitely less seriously attacked and that lilac-coloured varieties always appeared free from infection.

As indicated by investigations at Wisley, no variety of *Phlox paniculata* appears entirely to resist attack by this nematode, though varieties exhibit varying degrees of attractiveness and some few show little outward signs of infection. It was, for instance, possible to secure artificial infection of such non-attractive varieties as 'Antonin Mercie' and 'Widar', but they proved able to grow away from the attack, and the only visible damage was a cracking of the stem near the point of inoculation when subjected to artificial injection methods.

White varieties were stated, generally speaking, to

be the least liable to attack (Fox Wilson, 1924). A complete survey has now been made of the colour groups of some 460 varieties of herbaceous phloxes which were grown at Wisley, 1923–34, in order to ascertain whether there exists any correlation between colour of the flower and degree of attractiveness to the eelworm (Table 4, Text-fig. 1). The percentages of varieties which exhibited some degree of non-attractiveness varied from 18·35% (pink) to 4·75% (violet). Disregarding those that are grouped as being feebly infected, the percentage of those varieties which were moderately and severely infected ranged from 88·22% (scarlet) to 70·25% (white), which is in accordance with earlier results (Fox Wilson, 1924), indicating that phloxes with white flowers are, on the whole, least liable to severe infection.

Table 4

No. of vars. tested	Colour of flower	Not infected %	Infected %
42	Violet	5	95
34	Scarlet	9	91
37	White	13	87
85	Purple	14	86
104	Salmon	15	85
158	Pink	18	82

There is, unfortunately, an almost complete absence of data on the parentage of herbaceous phloxes, but amongst the comparatively few instances in which the origin of varieties can be traced, it is found that attractiveness to the nematode is transmissible to the offspring with seedlings and 'sports' from infected parent plants.

It is known that many stocks of herbaceous phloxes are infected with *A. dipsaci* despite statements to the contrary, and this is chiefly due to the fact that the infection is masked owing to favourable conditions of soil, site and climate in which such stocks are grown.

The author desires to express his sincere thanks to Dr T. Goodey for his unfailing help during the course of the investigations and for reading through the typescript; to his colleague, Mr F. C. Brown, for the photographs; and to his assistant, Miss P. Shilton, for working out the colour groups of phloxes and in preparing the chart dealing with this section of the investigations.

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EXPLANATION OF PLATES 7 AND 8

Plate 7

Fig. 1. Gladiolus corm. Temporary invasion of a phlox strain of *A. dipsaci* which persisted for some weeks before dying out.

Fig. 2. Narcissus bulb. (*a*) primary, and (*b*) secondary invasion centres of an onion strain of *A. dipsaci* injected directly into the bulb.

Fig. 3. Method of injecting suspensions of *A. dipsaci* into bulbs and corms.

Plate 8

Fig. 4. *Gilia coronopifolia* seedlings infected with a phlox strain of *A. dipsaci* in a starvation test.

Fig. 5. Method of testing the attractiveness of host plants to a biologic race of *A. dipsaci*. Seeds of four plant species and/or varieties sown together in one pan containing an onion race. The onion seedlings (1) were the only plants infected, while *Phlox Drummondii* (2), *Oenothera glauca Fraseri* (3), and *O. fruticosa Youngii* (4) were unaffected.

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The effect of a chemical soil sterilizing agent on the subsequent development of tomato plants

By J. H. Western and R. Stewart, *Agricultural Advisory Department, Manchester University*

(With Plate 9)

An account is given of the effect on the development of tomato plants of a chemical soil sterilizer containing orthodichlorbenzene and an emulsifying agent of the sulphonated oil type. The symptoms are described and an account is given of an experimental study of the effect of the treatment on growth and yield: the latter showed a considerable decrease. The injurious ingredient was shown to be orthodichlorbenzene.

During the summer of 1940 tomato plants with deformed leaves were received from three different growers and other cases were seen later. The plants were strong and vigorous but the leaves were coarse and leathery and leaflets showed varying degrees of distortion. The symptoms were more pronounced

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

FOX WILSON—The stem and bulb eelworm, *A. dipsaci*

towards the tops of affected plants, the lowermost leaves being normal, and virtually no fruit was harvested from them. Investigation revealed no cultural factors likely to account for the trouble and the plants showed no evidence of fungus or virus disease, but all the growers had used the same proprietary soil sterilizing chemical compound, the essential ingredients of which were orthodichlorbenzene and an emulsifying agent of the sulphonated oil type. To test the suspicion that this compound was connected with the abnormal growth of the plants, an experiment was carried out in 1941.

Experimental methods

Compost was made as recommended by Lawrence & Newell (1939) containing 2 parts sieved loam, 1 part coarse sand and 1 part sieved peat. The whole was well mixed on a concrete floor and divided into six equal portions which were treated with chemical sterilizing agents (Table 1) and left in heaps for one month. Each heap was well turned over from time to time. An equivalent amount of compost was mixed with a little soil from a house in which 'foot rot' and 'damping off' fungi were plentiful, and subjected to the same sterilizing treatments to test their efficiency. Under the experimental conditions no disease developed and both the inoculated and the control series may therefore be regarded as a single unit.

Table 1. *Sterilization of compost* (1 *gal./bush*)

Treatments	Strength
Control	Water
Formalin	2 % sol. in water
Proprietary (containing orthodichlorbenzene)	0·5 %* sol. in water
Cresylic acid	2·5 % ,,
Carbon bisulphide	1·33 % ,,

* Strength recommended by manufacturers.

Small seed boxes were filled with the appropriate soils (ten boxes of each soil) and placed in a glasshouse (temp. 55° F.). On 6 Mar. 1941, twenty-four Ailsa Craig tomato seeds were planted at regular intervals in each box and the boxes were covered with paper and glass until the emergence of the seedlings.

Results

The germination, with the exception of the cresylic acid series, was exceptionally good although the formaldehyde boxes were later by some days than the others. The orthodichlorbenzene product was among the best of the treatments and gave a rapid and even germination of 96·5 %. It is with the subsequent behaviour of these plants that this paper deals.

All the plants grew evenly and well and there was no sign of abnormality until 7 weeks after planting, when on every plant in the soil treated with the orthodichlorbenzene product the 5th and 6th leaves were distorted. Their laminae were greatly reduced and misshapen, often consisting of only a thin band of assimilating tissue bordering the veins (Pl. 9, fig. 2). All the leaves above the 5th and 6th were abnormal and the main stem failed to develop at the normal rate, the plants becoming stunted (Pl. 9, fig. 1*b*). These plants were carried on to maturity without heat. They grew vigorously and the new growth was progressively more normal in character until, in the uppermost portions of mature plants, apparently complete normality was reached in leaf, flower and fruit. The weight of fruit from abnormal plants, however, was appreciably less than from the controls (Table 2).

Table 2. *Yield of tomatoes per plant* (*lb.*)

Plant no.							
1	2	3	4	5	6	Mean	Difference
Control							
7·70	8·30	7·53	10·84	9·13	5·36	8·14	
Treated							
6·11	3·25	7·09	3·91	5·88	5·48	5·29	−2·85

An analysis of variance showed the standard error per plant to be 1·64 lb., i.e. the standard error for the mean of six plants is 0·67 lb. The difference between the two series of plants (2·85 lb.), being considerably more than $\times 3$ the standard error of the mean, is significant. The effect of the sterilizing treatment which produced visible foliage symptoms in early growth was thus reflected in a significant decrease in yield from the treated plants when these were grown to maturity under satisfactory conditions.

In 1942 an experiment was made to determine which ingredient of the sterilizing agent was responsible for the injury. Various solutions were prepared so that each represented the proprietary orthodichlorbenzene sterilizer minus one of its constituents. The solutions, together with the proprietary solution and pure water as a control, were applied at approximately comparable rates to batches of soil which were then potted and later set with tomato plants, one per pot.

The results were definite: with any treatment containing orthodichlorbenzene the growth of the plants was affected and the characteristic leaf distortion developed while other treatments produced normal plants. It seems clear that the ingredient in the proprietary sterilizer responsible for the harmful effect on tomato plants is orthodichlorbenzene and that the other ingredients are harmless.

Conclusion

Experiments demonstrated the harmful effect that a soil sterilizing agent containing orthodichlorbenzene can exert on tomatoes grown in soil treated with it.

In each of the last 3 years several cases were seen in which this particular injury followed the use of orthodichlorbenzene as a soil sterilizer and it is clear that this substance should not be added to soil intended for tomatoes. No experimental evidence was obtained as to the period necessary before treated soil loses its injurious properties but it seems likely to be considerable. In one instance a grower removed the soil from his house in the autumn moving it back again in the spring after exposure to the weather for the whole winter. When tomatoes were planted again they developed the abnormal symptoms although not to the same extent as in the previous year. In bad cases the fruits are affected and may assume bizarre shapes. It was thought possible that the chromosome complement might be modified in some way, but examination of the chromosomes of normal and abnormal plants failed to reveal any differences in their size or number.

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EXPLANATION OF PLATE 9

Fig. 1. Tomatoes 7 weeks after planting. (*a*) normal plant; (*b*) plant grown in soil treated with the orthodichlorbenzene product.

Fig. 2. The abnormal plant (Fig. 1*b*) seen from above.

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Studies in the mechanism of fungicidal action. VI. Water

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(With 2 Text-figures)

1. Theoretical deductions are made from the application of the theory of variability to hydration effects, concerning the effect on variability of a given population of spores to acid and alkali toxication, of varying the isotopic composition of the hydrogen. In the former case variability should decrease and in the latter increase with increasing deuterium content.

2. It is predicted that depolymerization of the water should decrease the variability of a given population of spores to any strongly hydrated ion, such as metallic cations.

3. Experimental techniques used in applying *in vitro* methods of exact toxicological investigation to solutions containing excess deuterium, and thus available in limited supply, are described.

4. The results of experiments designed to verify (1) are recorded. The expected trends are observed.

5. Exploratory tests bearing on (2) are described with inconclusive results. The importance of hydration effects in practice is stressed. Evidence is adduced that non-toxic electrolytes exert a direct desolvative action on hydrogen ions.

I. INTRODUCTION

Since all protective fungicides must act in an aqueous medium, it is important to know the effects of water on the toxic properties of solutes towards fungus spores. Excluding the grosser physical action of water (as a detergent, etc.) the effects may be due to chemical action (e.g. hydrolysis) on the active substance, or to solvation. This paper is confined to the latter effect to the investigation of which the theory of variability is especially adapted.

It follows from the theorem stated in equations (18) and (19) in the second paper in this series (Parker-Rhodes, 1942) that the variability of a given population of spores to any toxic ion or molecule susceptible of hydration will be greater, the greater the degree of hydration. It is not possible to predict the magnitude of this effect, but it will occur with most compounds usable as fungicides. Evidence is presented that the effect is sufficiently marked to be used to alter the slope of the probit-regression line, and thereby to modify the fungicidal properties of the compounds tested, at least under laboratory conditions.

To study the effect of differences in hydration without recourse to chemically extraneous addenda, use was made of the differing properties in this respect of the isotopes of hydrogen which in other matters (e.g. the toxicity of hydrogen ions *per se*)

Fig. 1

Fig. 2

may be presumed virtually identical. At high concentrations deuterium has a specific toxic action, but this has been found in the present case to be negligible (at subtoxic *p*H values) up to 70% D. The quantitative interpretation of the results is rendered difficult by the lack of published data on the solvation of the D^+ and OD^- ions; therefore, while themselves bearing on this point, the experiments here described are to be regarded more as indicating the kind of effects to be anticipated (which is the main point of practical importance) than as affording confirmation of theory.

II. THEORETICAL

(1) *Application of the theory of variability to solvated molecules*

Consider a molecule M solvated by a solvent A to give a complex MA_r. Considering all isomers of MA_r together, the following processes will tend to increase its concentration:

(*a*) decomposition: $MA_{r+s} \rightarrow MA_r + A_s$,

(*b*) association: $MA_{r-s} + A_s \rightarrow MA_r$,

and the following to diminish it:

(*c*) decomposition: $MA_r \rightarrow MA_{r-s} + A_s$,

(*d*) association: $MA_r + A_s \rightarrow MA_{r+s}$.

Let the probability that a given molecule will undergo (*a*) or (*c*) in unit time be $\phi'_{r,s}$ and $\phi'_{r-s,s}$ respectively, and the probability that a given collision between molecules will result in (*b*) or (*d*) be $\psi'_{r-s,s}$ and $\psi'_{r,s}$ respectively. Then the net rate of increase of $[MA_n]$ will be given by

$$\frac{dx_n}{dt} = \sum_1^n k\psi'_{n-r,r} a_r x_{n-r} - \sum_1^\infty (k\psi'_{n,r} a_r + \phi'_{n-r,r})\, x_n + \sum_1^\infty \phi'_{n,r}\, x_{n+r}, \quad (1)$$

wherein $x_s = [MA_s]$, $a_s = [A_s]$, and k is a constant depending on rate of collision. If M be very dilute, the a's may be presumed independent of the x's, and in that case we can put $k\psi'_{r,s} a_s = \psi_{r,s}$ (and $\phi'_{r,s} = \phi_{r,s}$). Now at equilibrium dx_n/dt is zero in so far as it is determinate; but there must be at least one x_n so small that its rate of change is indeterminate, as it is not a continuous variable, while for higher values of n, x_n will be effectively zero. Putting (1) equal to zero will therefore be valid for all values of n from 0 to $N-1$, but invalid if $n=N$. We thus derive N equations in the $N+1$ variables x_n ($n=0$ to N):

$$\sum_1^n \psi_{n-r,r} x_{n-r} - \sum_1^N (\psi_{n,r} + \phi_{n-r,r})\, x_n + \sum_1^{N-n} \phi_{n,r} x_{n+r} = 0 \quad [n=0 \text{ to } N-1]. \quad (2a)$$

An $(N+1)$th equation can be derived by putting ξ equal to the total concentration of M present (in all forms), whence

$$\sum_0^N x_r = \xi. \quad (2b)$$

The equations (2) are consistent and have a unique solution which may be written

$$x_r = \frac{\xi \Delta_{Nr}}{\Delta}, \quad (3)$$

wherein Δ stands for the determinant of the left-hand side of (2) and Δ_{ij} the co-factor of its element in the ith row and the jth column. (The elements given by $N=0$ are taken as forming the zeroth row.)

Taking care not to produce a statistically meaningless expression (see Parker-Rhodes, 1942, p. 133), the theory of variability can be applied to (3) giving

$$W_\alpha(\xi) = W_\alpha(x_r) + W_\alpha\left(\frac{\Delta}{\Delta_{Nr}}\right) + \alpha^2 W_\alpha(x_r)\, W_\alpha\left(\frac{\Delta}{\Delta_{Nr}}\right). \quad (4)$$

From here it remains to evaluate Δ/Δ_{Nr} in specified cases.

(2) *The case of hydrogen and hydroxyl ions in water*

Having regard to the mechanism of ionic transfer it is here a reasonable assumption that the probability ϕ_{0r} of an unhydrated ion splitting off from an r-hydrated complex is to a first approximation independent of r. Consider then the form of Δ:

$$\Delta \equiv \begin{vmatrix} \sum_1^N \psi_{0r} & -\phi_{01} & -\phi_{02} & \dots & -\phi_{0,N-1} & -\phi_{0,N} \\ -\psi_{01} & \sum_1^N (\psi_{1,r} + \phi_{1-r,r}) & -\phi_{11} & \dots & -\phi_{1,N-2} & -\phi_{1,N-1} \\ -\psi_{02} & -\psi_{11} & \sum_1^N (\psi_{2,r} + \phi_{2-r,r}) & \dots & -\phi_{2,N-3} & -\phi_{2,N-2} \\ \dots & \dots & \dots & \dots & \dots & \dots \\ -\psi_{0,N-1} & -\psi_{1,N-2} & -\psi_{2,N-3} & \dots & \sum_1^N (\psi_{N-1,r} + \phi_{N-1-r,r}) & -\phi_{N-1,1} \\ 1 & 1 & 1 & \dots & 1 & 1 \end{vmatrix}.$$

If, according to our assumption, ϕ_{0r} is the same for all r, all the terms in the expansion of Δ by its zeroth column will be zero, except the first and last (since the co-factors of the intermediate elements contain two rows, the top and bottom, which are multiples of one another). The expansion thus gives

$$\Delta = \sum_1^N \psi_{0r} \cdot \Delta_{00} \pm \Delta_{N0},$$

whence

$$\xi = x_0 \Delta / \Delta_{N0} = x_0 \left(\sum_1^N \psi_{0r} \frac{\Delta_{00}}{\Delta_{N0}} \pm 1 \right). \quad (5)$$

But inspection of Δ shows that if

$$\phi_{01}=\phi_{02}=\ldots=\phi_0,\ \Delta_{N0}/\Delta_{00}=\pm\phi_0;$$

furthermore our assumption also entails that

$$\psi'_{01}=\psi'_{02}=\ldots=\psi'_0;$$

hence
$$\xi=\pm x_0\left(1-\frac{k\psi_0'}{\phi_0}\sum_1^N a_r\right), \qquad (6)$$

the sign being + if N is odd and − if N is even.

To determine the a_r, consider the equilibria between the polymers of H_2O. If $[(H_2O)_n]=a_n$, these give equations

$$K_{11}a_1^2=a_2,$$
$$K_{12}a_1a_2=a_3=K_{11}K_{12}a_1^3,$$
$$K_{13}a_1a_3=a_4=K_{11}K_{12}K_{13}a_1^4,\ \ldots,\ \text{etc.}$$

and in general
$$a_r=K_ra_1^r. \qquad (7)$$

If now the ions can be absorbed only in the unhydrated form (4) becomes (putting $\alpha=0$ in accordance with experimental evidence)

$$W_0(\xi)=W_0(x_0)+W_0\left(1-\frac{k\psi_0'}{\phi_0}\sum_1^N K_ra_1^r\right). \qquad (8)$$

The further simplification of (8) depends on the experimental fact that $\log\xi$ does not depart significantly from normal distribution. Since the various terms of the polynomial on the right-hand side cannot all have the same distribution, there must be some approximation valid within the limits of sampling error of the type $k\sum_1^N K_ra_1^r\simeq Ca_1^s$ (C=a constant). Thus (8) becomes

$$W_0(\xi)\simeq W_0(x_0)+W_0\left(1-\frac{\psi_0'}{\phi_0}Ca_1^s\right). \qquad (9)$$

For the hydrogen ion, the constant ψ_0'/ϕ_0 must be relatively small, because, although higher hydrates exist, the mean hydration of H^+ is usually held to be less than 1 (see Britton, 1942). Thus, as s increases and a_1 concurrently decreases, the second term tends to $W_0(1)=0$, and increase in s will decrease $W_0(\xi)$. But the dissociation of H_3O^+ is much greater than that of H_2O, and we may therefore safely assume that for OH^-, ψ_0'/ϕ_0 is considerably greater. In this case (9) approximates to

$$W_0(\xi)=W_0(x_0)+s^2W_0(a_1), \qquad (10)$$

so that, unless a_1^s becomes much smaller, $W_0(\xi)$ will increase with s.

(3) *Qualitative prediction of effect of isotopic composition*

It is required to know, therefore, the effect of isotopic composition on the polymerization of water. It is generally agreed that both in solution (Bell & Wolfenden, 1935) and in the pure state (Raman spectrum data of Rao & Koteswaram (1938)), D_2O is more polymerized than H_2O. Therefore up to the point where deuterium itself becomes toxic, the variability to acid toxication should decrease with increasing D-content, while that to alkali-toxication should increase. Deuterium toxication will inevitably increase the variability where it is active.

(4) *The case of metallic ions*

In this case we make the assumption that the *M-A* bonds are much stronger than the *A-A* bonds, so that, for example, MA_2 will consist almost entirely of *A-M-A* molecules, the isomer *M-A-A* being negligible. Since only the latter can decompose in one step to give unsolvated M, all the ϕ_{0r} will be negligible in comparison with ϕ_{01}. Accepting this, the expansion of Δ by its zeroth column gives

$$\Delta=\sum_1^N\psi_{0r}\Delta_{00}+\psi_{01}\Delta_{10}-\psi_{02}\Delta_{20}+\ldots \pm\psi_{0,N-1}\Delta_{N-1,0}\pm\Delta_{N0}, \qquad (11)$$

but if $\phi_{0r}=0$ $[r\neq1]$, then $\Delta_{s0}=\pm\phi_{01}\Delta_{\substack{s0\\01}}$ $[s\neq0]$, and (11) becomes

$$\Delta=\sum_1^N\psi_{0r}\Delta_{00}\pm\phi_{01}\left(\sum_1^{N-1}\psi_{0s}\Delta_{\substack{s0\\01}}\mp\Delta_{\substack{N0\\01}}\right). \qquad (12)$$

Now the coefficient of ϕ_{01} in the above expression is simply the expansion of Δ_{01} by its own first column, with sign reversed, and thus it follows that

$$\frac{\Delta}{\Delta_{N0}}=\frac{\sum_1^N\psi_{0r}\Delta_{00}\mp\phi_{01}\Delta_{01}}{\Delta_{N0}}.$$

It will be observed that since the ϕ and ψ are all positive, the diagonal elements of Δ are algebraically the largest by a factor $\sim N$; Δ_{00}, having $N-1$ of these elements, will therefore be greater than Δ_{01}, which has $N-2$ of them (and ϕ_{01} instead of the first), to the same order. Hence

$$\sum_1^N\psi_{0r}\Delta_{00}\sim N^2\phi_{01}\Delta_{01}\gg\phi_{01}\Delta_{01},$$

and so far any probable value of N

$$\frac{\Delta}{\Delta_{N0}}\simeq\frac{\Delta_{00}}{\Delta_{N0}}\sum_1^N\psi_{0r}. \qquad (13)$$

But the assumption made previously that

$$\psi'_{01}=\psi'_{02}=\ldots=\psi'_{0r}=\psi'_0$$

will be equally valid as in the former case, since two A_r molecules cannot co-ordinate to M simultaneously. Therefore

$$\sum_1^N\psi_{0r}=k\psi'_0\sum_1^N K_ra_1^r,$$

and by applying the theory of variability to (13) with the same approximation as before we get

$$W_\alpha\left(\frac{\Delta}{\Delta_{N0}}\right)\simeq W_\alpha\left(\frac{\Delta_{00}}{\Delta_{N0}}\right)+s^2W_{\alpha/s}(a_1) +\alpha^2s^2W_{\alpha/s}(a_1)\,W_\alpha\left(\frac{\Delta_{00}}{\Delta_{N0}}\right), \qquad (14)$$

this, reduced to the case of $\alpha=0$, gives for comparison with (10)

$$W_0(\xi) \simeq W_0(x_0)+s^2W_0(a_1)+W_0\left(\frac{\Delta_{00}}{\Delta_{N0}}\right), \quad (15)$$

without the possibility of reverting to an approximation of the type $W_0(\xi) \simeq W_0(x_0)$.

(5) *The effect of neutral electrolytes*

It is generally accepted that electrolytes tend to depolymerize their solvent water (see Rao, 1934). Thus they may concurrently tend to desolvate aqueous ions and perhaps also to weaken the solvate bonds of other molecules or ions. In the absence of a direct desolvative action (reduction in ψ_0'/ϕ_0) electrolytes will increase rather than decrease the variability to hydrogen ions (as follows from (9)), whereas if ψ_0'/ϕ_0 is markedly reduced the variability will be decreased. In the case of hydroxyl ions and metallic salts the only effect will be a decrease of variability, which, however, would be enhanced by any direct desolvative action.

Whether the latter occurs is thus of some interest, and experimental evidence, from the toxic behaviour of acids in the presence of their salts, can be adduced. Experiment must also decide whether these effects can be of practical significance in the action of fungicides.

It follows further from (15) that, since the last two terms depend only on the state of the solvent, equal quantities of a given depolymerizing agent will exert a numerically equal effect on the variabilities to all fungicidal compounds which act at such dilutions that their own effect on the solvent can be ignored. Hence the magnitude of the effect on the L.D. 98, for example, assuming that the L.D. 50 is unaffected, will be the greater, the less is the intrinsic variability $W_0(x_0)$.

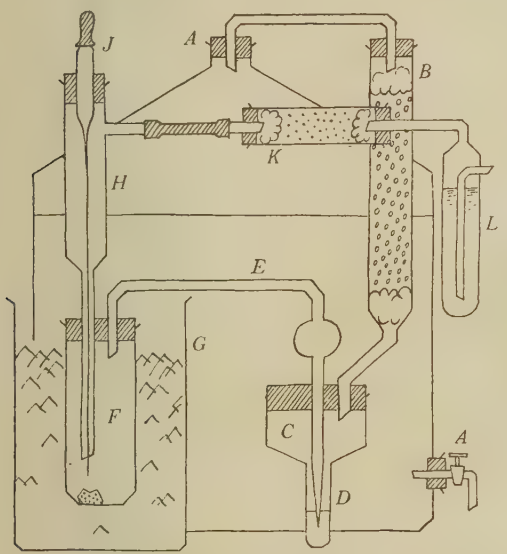

Fig. 1. Apparatus for the preparation of deuterium chloride; for explanation see text.

III. EXPERIMENTAL

(1) *Chemical materials*

The following compounds were used in the main investigations: hydrogen chloride, sodium hydroxide, deuterium chloride, sodium deuteroxide, deuterium oxide (and mixtures of these). Natural water was used as a control medium, and the non-toxicity of sodium chloride at the concentrations used was demonstrated.

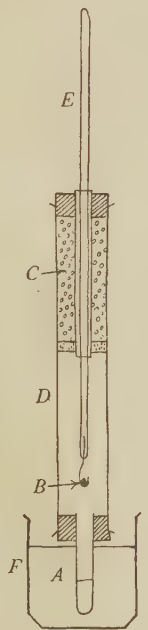

Fig. 2. Apparatus for the preparation of sodium deuteroxide; for explanation see text.

The deuterium oxide was prepared by the Norsk Hydroelektrisk Kvaelstofaktieselskab and stated to contain 99·6 % D_2O, on the assumption that 100 % D_2O has a sp. gr. $d_4^{22}=1{\cdot}10541$.

The deuterium chloride was prepared by the action of deuterium oxide on a fresh sample of phosphorus pentachloride:

$$PCl_5+D_2O \rightarrow PCl_3O+2DCl.$$

The reaction was carried out in the apparatus shown in Fig. 1. *A* is an aspirator by means of which a current of air, freed from H_2O by passing through

conc. H_2SO_4 in *L* and dry P_2O_5 in *K*, can be passed through the reaction vessel *F*, carrying the liberated DCl into *C*, where it is dissolved in deuterium oxide contained in the narrow part *D* (capacity *c.* 1·5 c.c.). *B*, containing $CaCl_2$, served to keep the air in *C* dry; the bulb in *E* avoided sucking back of the D_2O in *D*. In use, 0·2 c.c. of D_2O were introduced into *D* and a small lump of PCl_5 into *F*, *H* being closed by a rubber cork. After passing the air through for a few minutes the micropipette *J*, containing 0·02 c.c. D_2O, was fixed by its rubber sleeve into *H*, and its contents projected on to the PCl_5 in *F*. The latter vessel was immersed in freezing mixture in *G* in order to avoid vaporization of the oxychloride. Air was passed continuously for 15 min., after which time *C* was removed and stopped at the neck of *D*. 0·04 c.c. of solution from this vessel, diluted to 25 c.c. with distilled water, were titrated against standard NaOH solution.

The sodium deuteroxide was prepared in the apparatus shown in Fig. 2 by the action of the metallic sodium in *B* (carefully freed from paraffin with filter paper) on deuterium oxide, of which 0·2 c.c. were contained in *A*. The contents of the vessel *D* were kept dry by means of $CaCl_2$ in *C*. In use, *A*, having been fitted in place of a rubber cork at the base of *D* as shown, was immersed in cold water in *F*, and *B* lowered into it by means of the glass rod *E*: 10 min. were allowed to complete the reaction, after which *A* was removed and stopped. The concentration of the resulting solution was established by titration of a diluted sample against standard HCl.

In these operations all apparatus was previously dried at 60° C. overnight. Stock solutions in deuterium oxide were kept in glass vessels with rubber stoppers sealed with soft wax. Solutions of intermediate isotopic composition were prepared by mixing known volumes of the above with ordinary HCl and NaOH solutions.

(2) *Biological materials*

The test organism was *Macrosporium sarcinaeforme* from the same stock as used in the previous work of this series: the methods of culture were the same throughout.

(3) *Experimental technique*

The following alterations were made in the technique hitherto employed (Parker-Rhodes, 1941) in order to reduce the volume of medium required:

(*a*) The number of drops of spore suspension was 3 per replicate instead of 5.

(*b*) The volume of each drop was half that used previously.

(*c*) A more concentrated spore suspension was used; and every normal spore in every drop was counted, instead of aiming at a fixed number. There were usually from 300 to 500 spores available per replicate, as against 500 in the standard technique.

(*d*) The toxicant solutions were made up in ignition tubes of *c.* 0·5 c.c. capacity; each contained 0·04 c.c. of solution.

(*e*) All solutions containing supernormal quantities of deuterium were handled in special micropipettes kept in the presence of $CaCl_2$ and dried at 60° C. after use.

The experimental drops were exposed to air saturated with ordinary water vapour as in previous work. Although this, reacting with the D_2O, effects some protization, this effect was ignored in the calculations since it could not be allowed for quantitatively. However, since temperature and duration of test were uniform throughout the experiments, all D concentrations would be reduced in approximately the same ratio.

In dealing with ordinary HCl and NaOH solutions the ordinary technique was used, as allowing greater accuracy.

(4) *Presentation of results*

The results are given in Tables 1–3; here x denotes the logarithm of the hydrogen or hydroxyl ion concentration to the base *e*, in arbitrary units. Owing to fluctuations in the modal tolerance, adjustments had to be made to the x values to bring the results of different replicates into alignment; since the index of variation was zero, this could be done by adding a constant to all the x-values of each divergent replicate; the figures given are the adjusted values. q denotes proportional mortality, and N the total number of spores on which the count was made; no corrections to N were necessary on account of the controls since (with certain exceptions discussed below) these all gave germination above 90%.

In Table 3, w denotes the reciprocal variance of the estimate $U_0^{-\frac{1}{2}}$ given in column 3, calculated from the observations by the formula $w = \frac{nA}{\chi^2}$ (for notation see Parker-Rhodes, 1942). The figures in columns 1, 3 and 4 enable the regression of $U_0^{-\frac{1}{2}}$ on proportion of deuterium to be calculated.

Although with the hydrogen ions direct comparison of different U_0 values is not very instructive, the regression coefficient is highly significant up to 60% D [$P = 0{\cdot}015$]; but the last figure (70% D) lies significantly below the line. No other value departs significantly from the line, nor do the figures for 30, 40, 50 and 60% D show any regression. The figure for 70% D lies below the mean of these four, but the difference is non-significant ($P = 0{\cdot}06$). With hydroxyl ions the difference between U_0 for 0 and 50% D is very highly significant [$P < 0{\cdot}01$].

(5) *The toxicity of deuterium oxide* per se

It was found that although germination in 50% D_2O was entirely normal, none occurred in 95% D_2O. Even a 10 min. exposure to this concentration effected a delay of about 24 hr. in the germination of

TABLE 1. *Effect of isotopic composition of hydrogen ions on their toxicity to* Macrosporium

Vol. D_2O/vol. H_2O ...	0 : 10		1 : 9			2 : 8			3 : 7		
Proportion hydrogen atoms of mass 2 ...	0·0002			0·0994			0·1987			0·2983	
	x	*q*	*N*	*x*	*q*	*N*	*x*	*q*	*N*	*x*	*q*	*N*
	0·000	0·000	750	0·000	0·000	302	0·000	0·013	541	0·000	0·043	186
	0·095	0·018	400	0·223	0·044	200	0·223	0·044	460	0·223	0·267	337
	0·223	0·027	700	0·446	0·023	266	0·446	0·126	450	0·446	0·847	331
	0·382	0·074	400	0·590	0·307	400	0·669	0·619	500	0·669	0·953	230
	0·446	0·061	750	0·669	0·175	375	0·892	0·849	300	0·892	1·0000	200
	0·669	0·184	750	0·892	0·590	248	1·115	0·9641	500			
	0·956	0·878	300	0·995	0·802	600	1·338	0·9943	371			
	1·115	0·9684	500	1·115	0·959	450						
	1·243	0·954	350	1·218	0·909	600						
	1·338	0·9959	750	1·483	0·9829	600						
	1·530	0·9694	166	1·664	1·0000	300						
	1·568	1·0000	500									

Vol. D_2O/vol. H_2O ...	4 : 6		5 : 5			6 : 4			7 : 3		
Proportion hydrogen atoms of mass 2 ...	0·3981			0·4981			0·5967			0·6967	
	x	*q*	*N*	*x*	*q*	*N*	*x*	*q*	*N*	*x*	*q*	*N*
	0·000	0·022	500	0·000	0·025	178	0·000	0·000	650	0·000	0·047	980
	0·067	0·186	309	0·056	0·000	250	0·223	0·051	650	0·223	0·595	764
	0·290	0·705	325	0·077	0·095	348	0·446	0·616	600	0·446	0·748	770
	0·446	0·954	327	0·223	0·110	448	0·669	0·959	600	0·669	0·9884	388
	0·513	0·946	258	0·279	0·025	326	0·892	0·9881	750			
	0·669	0·9894	300	0·300	0·139	417						
	0·736	0·9947	223	0·446	0·632	281						
	0·892	1·0000	300	0·502	0·900	369						
				0·523	0·661	367						
				0·669	0·937	201						
				0·725	0·939	250						
				0·746	0·954	246						
				0·892	0·9946	390						
				0·948	0·9965	288						
				0·969	0·9849	300						

TABLE 2. *Effect of isotopic composition of hydroxyl ion on toxicity to* Macrosporium

Vol. D_2O/vol. H_2O...	0 : 10		5 : 5		
Proportion hydrogen atoms of mass 2 ...	0·0002			0·4981	
	x	*q*	*N*	*x*	*q*	*N*
	0·000	0·034	371	0·000	0·116	226
	0·223	0·078	500	0·223	0·032	319
	0·446	0·210	500	0·446	0·245	222
	0·669	0·554	500	0·669	0·197	226
	0·892	0·828	500	0·892	0·359	180
				1·115	0·405	230
				1·338	0·867	253
				1·561	0·658	275
				1·784	0·806	281

the same spores subsequently exposed to 50% D_2O. This toxic (apparently fungistatic) action of deuterium oxide was not investigated in detail; it was detectable as a slight reduction in percentage germination in 60 and 70% D_2O, but up to this level it failed to appear in the presence of subtoxic concentrations of hydrogen ions; for this reason the *q* values in these two cases were calculated using the most dilute acid solution tested in place of the true controls. At higher D concentrations the figures were judged to be unreliable.

TABLE 3. *Calculations from Tables 1 and 2*

Ion	Composition (atoms D per total hydrogen)	Variability U_0	Variability $U_0^{-\frac{1}{3}}$	Weight *w*
H^+ and D^+	0·0002	0·071	3·76	1·25
	0·0994	0·081	3·51	1·27
	0·1987	0·063	3·97	3·62
	0·2983	0·0246	6·36	1·94
	0·3981	0·0202	7·03	1·32
	0·4981	0·0257	6·23	1·48
	0·5967	0·0194	7·19	0·75
	0·6967	0·0305	5·72	17·00
OH^- and OD^-	0·0002	0·078	3·59	14·65
	0·4981	0·475	1·45	110·53

In making up test drops in 60 and 70% D_2O solutions, the spore suspension was made up in 40% D_2O and the toxicant solutions contained 80 and 99% D_2O respectively. (The percentages stated in the text are approximate only; accurate figures are given in the tables.)

IV. Discussion

The results bear out in a quantitative fashion the prediction, made on the basis of theory, that the variability of a given population of spores towards acid toxication should decrease with increasing concentration of deuterium, whereas towards alkalies the opposite effect should appear. Furthermore, the expected increase of variability occurs where the toxic action of deuterium oxide itself becomes operative.

Table 4. *Effect of electrolytes on the toxic properties of the cupric ion*

First experiment

Log. conc. Cu^{++}	$CuSO_4+M/80$ NaCl		Control	
x	q	N	q	N
0·00	0·000	385	0·038	413
0·41	0·001	339	0·100	370
0·81	0·116	400	0·359	400
1·22	0·797	331	0·813	390
1·62	0·9963	284	0·946	234

Second experiment

Log conc. Cu^{++}	$CuSO_4+M/80$ K_2SO_4		Control	
x	q	N	q	N
0·000	0·000	284	0·109	209
0·272	0·012	212	0·157	327
0·575	0·242	275	0·452	331
0·861	0·412	340	0·855	311
1·150	0·946	325	0·891	423
1·437	0·9640	294	0·926	325

The support thus lent to theory encourages the belief that the hydration effects predicted in the case of heavy metals should be observable. Furthermore, the magnitude of the effect, as judged by the change in variability to alkalies on passing from the natural hydrogen to that containing 50% D, is likely to be large enough to have some importance. For this reason two attempts were made to affect the slope of the regression line of probit mortality against logarithmic concentration of copper by the use of cupric sulphate, alone, and in presence of additional electrolytes. The latter, as previously indicated, ought to steepen the slope.

The figures are recorded in Tables 4 and 5. The effect was appreciable; that for NaCl was fully significant, that for K_2SO_4 not significant. The interpretation in the former case is complicated by the possibility of formation of complex ions of the type $[CuCl_3]^-$, so that these experiments cannot be regarded as conclusive. The object of recording the results is to draw attention to the way in which toxicologically neutral substances may affect the performance of fungicides with which they may be incorporated, whether as spreaders, wetters, etc., or for their specific chemical effects, and to the probable importance of hydration in the genesis of such effects.

To obtain evidence whether such action of electrolytes as is demonstrated in these results can be interpreted merely by their depolymerizing action, or whether, as postulated in § II (5), they also exert a desolvative action on the ions, the variability of the spores to hydrochloric acid with and without added $M/40$ NaCl was estimated. A marked reduction in the variability was effected by the salt (though exact figures could not be obtained since the regression line had zero degrees of freedom in the second case), indicating that desolvative action (reduction in ψ_0'/ϕ_0) was operative. Presumably this also applies (in less degree) to the case of the cupric ion.

Table 5. *Calculated from Table 4*

	U_0	$U_0^{-\frac{1}{3}}$	χ^2/nA
First experiment:			
$CuSO_4$+NaCl	0·042	4·85	0·201
Control	0·179	2·36	0·049
Second experiment:			
$CuSO_4+K_2SO_4$	0·071	3·76	0·363
Control	0·188	2·30	0·115
From Parker-Rhodes (1941):*			
Thioureocuprous ion	0·106	—	—
Cupric ion	0·361	—	—

* Figures expressed in terms of natural logs.

Meanwhile, the main significance of the results may be summarized as follows: (*a*) they show that hydration effects can be of significant magnitude in toxicological work, (*b*) they lend further support to the theory of variability as an analytical method, (*c*) they point the way to a wide new field for quantitative investigation of importance in fungicidal work, in which the methods developed in this series of papers may be of use.

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Studies upon the copper fungicides
VI. The solution of copper from Bordeaux and Burgundy mixtures

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(With 1 Text-figure)

The soluble copper in freshly prepared 4:4:50 Bordeaux mixture was of the order of 7 p.p.m. but on standing in absence of air this fell to 0·7 p.p.m. after 10 days. The dried deposit on glass plates yielded about 0·5 p.p.m. of copper to water. With increasing alkalinity, the soluble copper in freshly prepared Burgundy mixtures decreased to a minimum of about 3 p.p.m. and then rose again. This effect was not observed using dried deposits which yielded consistently less copper to water. The amounts of copper dissolved by water from dried 4:4:50 Bordeaux deposit on runner bean leaves were slightly greater than from the same deposit on glass plates. The results cannot be taken as positive evidence that leaf excretions are involved in the liberation of soluble copper since detached leaves were used and the influence of bacteria could not be eliminated.

Suspensions of *Neurospora sitophila* spores and the filtrates therefrom dissolved copper from dried 4:4:50 Bordeaux deposit in excess of the amount dissolved by water, though these quantities, as well as the amounts of soluble solids in spore filtrates, were appreciably less than were found by other workers in a similar investigation. Steam sterilization of spore filtrates had little effect on their capacity to dissolve copper from dried 4:4:50 Bordeaux. The temperature at which spores were suspended in water was a factor, though the substances capable of dissolving copper were rapidly yielded to water. The nutrient agar on which the fungus was grown influenced the amount of soluble solids in the spore filtrate and the capacity of these filtrates to dissolve copper from dried Bordeaux deposit.

Study of the solvent properties of solutions of pure chemical substances of many types showed that copper could only dissolve from dried 4:4:50 Bordeaux deposit by a mechanism involving complex formation. Active substances included amino-, hydroxy-, and certain dicarboxylic acids and their salts, all possible constituents of spore exudate. The results obtained with carbohydrates indicated that such substances are not involved in the liberation of soluble copper from this fungicide.

A hypothesis on the mechanism of the fungicidal action of copper, based on the results obtained, is presented. This assumes that copper dissolves from the deposit under the influence of excretions from the fungus spore and that the cupri-complexes produced are the means by which soluble copper is transported to the spore wall. Dissociation of these complexes then make it possible for the active toxicant to be removed by the spore, thus enabling any reversible reactions involved to continue.

Although usually regarded as insoluble, the moist and dry precipitates of Bordeaux and Burgundy mixtures dissolve slightly in water and, possibly, the small quantities of copper in solution play an important part directly or indirectly in the fungicidal action of these substances. The amounts of soluble copper in the supernatant liquids of various Bordeaux mixtures were determined by Hockenyos (1931) and in two highly alkaline Burgundy mixtures by Pickering (1909).

The amount of copper yielded to distilled water by 4:4:50 Bordeaux deposit was found by McCallan & Wilcoxon (1936) not to exceed 0·3 p.p.m., a quantity not toxic to most fungus spores: e.g. with *Macrosporium sarcinaeforme*, Martin *et al.* (1942) showed in tests with copper sulphate solutions that at the L.D. 50, the maximum concentration of soluble copper was 2·7 p.p.m. Barker & Gimingham (1911) pointed out that increased amounts of soluble copper might be produced from dried Bordeaux deposit by

(1) the chemical action of gases present in the air, especially carbon dioxide; (2) the action of the host plant, and (3) the action of the fungus. Another hypothesis assumes that the small amounts of copper dissolved from the fungicide by water are removed from solution by absorption into the spore, more copper dissolves from the fungicide to maintain the solution equilibria, and further quantities of soluble copper thus become available. Absorption of soluble copper proceeds in this way until a toxic amount is reached. Each of these hypotheses has received attention. (For review of earlier work, see McCallan, 1930.)

There is little published evidence that the host plant can influence the solution of copper from Bordeaux mixture (McCallan, 1930), but whether this occurs or not, the fungicide on sprayed glass slides is toxic to spores, under conditions in which the effects of leaf excretions and weathering are eliminated. Wilcoxon & McCallan (1938) indicated that weathering might be an important factor in the fungicidal and phytocidal action of Bordeaux mixture in the field, soluble copper exceeding toxic concentrations being released from a deposit subjected to leaching action of rain.

Experiments based on the suggestion that excretions or lysates from the fungus and its spores are involved in fungicidal action gave encouraging results. McCallan (1930) presented data favouring this hypothesis and McCallan & Wilcoxon (1936) showed that the copper dissolved from dried Bordeaux deposit by water in which spores of different species of fungi had been suspended, was appreciably greater than that dissolved by distilled water.

Spore excretions consist mainly of solutions of organic substances some of which are able to take copper into solution from the insoluble fungicide, and the nature of these substances must be known before the complete mechanism of fungicidal action is understood. A solution obtained by suspending spores of *Neurospora sitophila* in water was examined chemically by McCallan & Wilcoxon (1936) and a similar study is in progress in this Laboratory. By such analytical methods substances present in the exudate may be identified and the capacity of their solutions to dissolve copper ascertained. The analysis of the mixture making up the spore exudate cannot be exhaustive owing to the small quantities of material available. One object of the present work was to determine by direct means what types of organic substance are capable of liberating copper from a successful copper fungicide, viz. 4:4:50 Bordeaux mixture.

The types of compound which produce soluble copper from dried Bordeaux would probably be active with other insoluble copper fungicides. Sessions (1936) suggested that the effect was closely related to the acidity of cell sap of the leaves, but McCallan & Wilcoxon (1936) showed that sodium malate, glycine and aspartic acid possessed this property, and observed that malate was present in the exudate from *N. sitophila* spores. The presence of amino acids was also indicated.

In their action on insoluble compounds like cupric oxide or hydroxide, organic acids should dissolve an equivalent amount of copper providing the resulting salt is soluble enough. Further, solutions of the salts of some organic acids can dissolve copper from such substances, the copper entering the solution as a complex ion, an effect which might also occur with certain basic or neutral substances.

Dried alkaline Bordeaux precipitates differ from ordinary insoluble copper derivatives in the presence of much calcium carbonate in the deposit, but this does not interfere with the action of Bordeaux so that it may be assumed that toxic concentrations of soluble copper are yielded in presence of calcium carbonate.

To determine how copper enters solution from dried Bordeaux, shaking experiments were done using solutions of pure organic substances of many types, irrespective of whether they are likely to occur in spore extracts.

Experimental

Materials and methods

The preparation of Bordeaux and Burgundy mixtures. 4:4:50 Bordeaux mixture which was used throughout this work was prepared from equal weights of A.R. $CuSO_4.5H_2O$ and finely ground carbonate-free calcium hydroxide. Burgundy mixtures were made from stock solutions of A.R. copper sulphate and sodium carbonate at equivalent strengths.

Dried deposits were obtained by spraying the fungicide on to 14 cm. diam. glass Petri dishes, using Evans & Martin's (1935) atomiser. Each dish was sprayed for a timed interval at 2 atm. pressure and allowed to dry for at least $4\frac{1}{2}$ hr. The filtrate of a spore suspension or 50 ml. water was introduced, the cover fitted and the dish agitated on a mechanical shaker with a rotary motion for 17 hr. The liquid was then centrifuged at 2500 r.p.m. for 10 min. and samples pipetted for analysis. In all experiments, undissolved copper was present in the residual deposit.

The preparation of solutions for solvent experiments. All acids and their salts were used at a concentration of $N/1000$ and other substances as stated. Where possible $N/1000$ solutions were made by diluting standardized $N/10$ solutions. In preparing $N/1000$ solutions of sodium salts, standardized solutions of the acids were exactly neutralized with NaOH and diluted. Solutions of the sodium salts of amino acids were prepared by adding the theoretical quantity of carbonate-free NaOH solution to a weighed quantity of the acid. This solution was then diluted to $N/1000$. Pure chemicals, recrystallized where necessary, were

used throughout and glass-distilled water was employed in making up all solutions.

6-methyl-3-*iso*-propylsalicylic and 2-methyl-5-*iso*-propyl-4-hydroxybenzoic acids were prepared as described by Kobek (1883).

The estimation of soluble copper. Micro-estimations of soluble copper were made by the diethyldithiocarbamate method (Callan & Henderson, 1929). The golden yellow copper salt was developed in ammoniacal solution and extracted with amyl alcohol (McFarlane, 1932).

Long narrow (10 × 0·7 in.) tubes fitted with ground-glass stoppers were used in the analysis. Each had a capacity of 30 ml. and was graduated at the 20 ml. mark.

A standard solution, 1 ml. of which contained 0·01 mg. Cu, was prepared by diluting × 10 a solution containing 0·3928 g. A.R. $CuSO_4.5H_2O$ in 1 l. of water.

To determine what quantity of an unknown solution to use for analysis, the liquid was added from a graduated pipette to a test-tube containing the reagent until the depth of colour approximated to that given by a known amount of standard solution. The volume required was then pipetted accurately into an analysis tube. The solution was diluted to about 15 ml., 0·5 ml. A.R. conc. HCl added and the tube shaken. 1·5 ml. A.R. 0·88 NH_4OH was added followed after shaking by 0·5 ml. 2% freshly filtered solution of sodium diethyldithiocarbamate. The contents were made up to 20 ml. with water, the tube shaken and 5 ml. A.R. amyl alcohol added from a pipette. After shaking and allowing to stand for at least 10 min. the amyl alcohol layer was removed by a teat pipette and filtered through an acid-washed filter paper into a dry test-tube. The depth of colour was then matched against an amyl alcohol solution prepared in an exactly similar way from a known volume of the standard using a colorimeter fitted with micro cups.

The error of this method tested over a range of solutions containing 5–20 p.p.m. of copper using a 10 p.p.m. standard was within ±2·5%, and the recovery of copper added to a sample of supernatant liquid from 4:4:50 Bordeaux was within 2%. (All apparatus was washed twice with conc. HCl, then with water, distilled water and glass-distilled water. Frequent 'blank' determinations using all the reagents failed to reveal the presence of copper even when only 2 ml. amyl alcohol were used for extraction.)

The collection of fungus spores. Spores of *N. sitophila* from an original culture kindly supplied by Dr S. E. A. McCallan of New York, U.S.A., were sown on solid nutrient agar in glass jars, and incubated at 25° C. The spores were removed after 7 days using McCallan & Wilcoxon's (1936) vacuum technique. The suspension was at once filtered through boiled-out cheese cloth to remove mycelium and allowed to stand, usually for 24 hr., during which spore counts were made in random samples using a Thoma haemocytometer. The spores were then filtered off, the filtrates being used as solvents in shaking experiments at laboratory temperature (12–16° C.). Nutrient agars used were 2 and 5% malt extract, potato dextrose, containing the extract of 300 g. minced potatoes and 50 g. dextrose/l., and a bread-mulch agar containing 15% moist bread.

RESULTS

The production of soluble copper from Bordeaux and Burgundy mixtures

(i) *By water*

(*a*) *Bordeaux mixture.* 500 ml. Bordeaux mixture was made up in a glass-stoppered measuring cylinder, shaken vigorously for 5 min. and allowed to stand. The precipitate slowly settled leaving a water-clear supernatant liquid from which samples were pipetted at intervals for analysis, care being taken not to disturb the precipitate. The cylinder was kept stoppered throughout except when samples were being taken. The amounts of supernatant liquid removed were small compared with the bulk and would not materially affect the composition of the mixture (Table 1).

TABLE 1. *Soluble copper in Bordeaux supernatant*

Time (hr.) ...	4	8	12	24	48
Sol. Cu (p.p.m.) (av. of duplicates)	6·89	5·87	5·20	4·62	3·83
Time (hr.) ...	72	96	120	240	
Sol. Cu (p.p.m.) (av. of duplicates)	2·32	1·51	1·43	0·69	

The copper dissolved from dried Bordeaux deposit when 50 ml. quantities of water were stood overnight in sprayed dishes at 3, 14 and 25° C. was 0·32, 0·36 and 0·36 p.p.m. In two shaking experiments done at the same time at 25° C., 0·44 and 0·43 p.p.m. Cu were brought into solution. The average of 26 determinations made by shaking water in sprayed dishes at laboratory temperature was 0·52 p.p.m.

In an experiment using the dried deposit of the more alkaline Bordeaux made from equal parts of $CuSO_4.5H_2O$ and CaO, 0·404 p.p.m. Cu were taken into solution by water.

(*b*) *Burgundy mixture.* By varying the amounts of carbonate, 100 ml. quantities of various Burgundy mixtures were made up in stoppered measuring cylinders. After shaking, the mixtures were centrifuged at 2500 r.p.m. for 5 min., copper determined in the supernatant liquids, and the *p*H taken (Table 2).

Burgundy mixtures sprayed on to glass and allowed to dry yielded much less copper to water (Table 3).

TABLE 2. *Soluble copper in Burgundy supernatants after centrifuging*

'*x*' in formula 4:*x*:50	4·59	5·50	6·42	7·34	7·80	8·26
*p*H	6·05	6·49	7·06	8·28	8·72	8·81
Cu (p.p.m.)	91·53	29·37	10·71	3·61	3·38	3·71
'*x*' in formula 4:*x*:50	8·44	8·63	8·77	9·19	11·00	15·77
*p*H	8·98	9·00	9·08	9·16	9·51	9·68
Cu (p.p.m.)	3·93	4·29	4·24	4·54	5·68	6·90

TABLE 3. *Copper dissolved from dried Burgundy deposits*

'*x*' in formula 4:*x*:50	5·96	6·42	6·88	7·34
Cu (p.p.m.)	1·35	1·05	0·97	0·85
'*x*' in formula 4:*x*:50	7·80	8·26	8·71	9·18
Cu (p.p.m.)	0·82	0·81	0·85	0·77

(ii) *By leaf exudates*

Runner bean plants (*Phaseolus multiflorus*), whose growing points had been removed, were selected with two nodal leaves approximately equal in size and visibly free from injury. The leaves were sprayed with Bordeaux mixture for 15 sec., one on the upper and the other on the lower surface. After allowing to dry for at least 4½ hr. they were detached and their surface areas determined. The leaves were then placed in 14 cm. diam. Petri dishes with 60 ml. water, glass triangles being used to hold the leaves below the surface so that mechanical injury during shaking was avoided. The distal end of the petiole was kept well clear of the water. The dishes were shaken for 17 hr. together with a sprayed dish containing water only. After centrifuging, the *p*H of the solutions was taken and soluble copper determined (Table 4).

TABLE 4. *Copper dissolved from 4:4:50 Bordeaux deposit on runner bean leaves*

Treatment	Leaf area sq. cm.	Cu dissolved (p.p.m.) (av. of duplicate)	Cu dissolved/100 sq. cm. sprayed leaf surface p.p.m.	*p*H
1 leaf (upper surface)	101	1·31	1·30	6·99
1 leaf (lower surface)	92	1·23	1·43	7·02
2 leaves (upper surface)	134	1·15	0·86	7·11
2 leaves (lower surface)	131	0·98	0·75	7·17
Blank	—	0·85	—	—

In another experiment, a leaf of area 89 sq. cm. sprayed on the lower surface yielded 1·26 p.p.m. soluble copper compared with a 'blank' of 0·54 p.p.m. The other leaf at this node, sprayed on the upper surface, became displaced during shaking so that the petiole was submerged, and on testing the solution about 4 p.p.m. of copper was detected. Further tests showed that sap expressed from stems of runner bean leaves dissolved much copper from dried Bordeaux deposits.

When ten detached leaves were immersed, except for the petioles in 500 ml. water for 79 hr. in the dark, a cloudy solution resulted which readily dissolved copper from dried Bordeaux deposit. The solution was acid and heavily contaminated with rod-shaped bacteria.

(iii) *By fungus spores*

That spores should be collected free from contamination by the nutrient medium was stressed by McCallan & Wilcoxon (1936) who found potato dextrose agar washings to dissolve copper from dried Bordeaux. In the present work when a slant of 2% malt agar was inverted ten times with water, the resulting liquid dissolved 2·51 p.p.m. Cu from dried Bordeaux, a figure much higher than that for water alone.

(*a*) *Copper dissolved by spore suspensions.* Dilutions were made from a mycelium-free suspension of 1656×10^6 spores in 200 ml. water and shaken in sprayed dishes. After centrifuging, the quantities of copper dissolved were determined (Table 5 and Fig. 1).

TABLE 5. *Copper dissolved by spore suspensions*

No. of spores in 50 ml. suspension (millions)	Cu dissolved (av. of duplicates) mg.
82·8	0·049
165·6	0·074
248·4	0·091
331·2	0·124
414·0	0·158

(*b*) *Copper dissolved by spore filtrates.* The spores in a suspension of known concentration were filtered off after 24 hr. and dilutions of the filtrate were shaken in sprayed dishes. The amounts of soluble copper determined are given in Table 6 and Fig. 1.

TABLE 6. *Copper dissolved by spore filtrates*

No. of spores in 50 ml. (millions)	Cu dissolved (av. of duplicates) mg.
0	0·017
54·1	0·025
108·2	0·036
216·5	0·056
433·0	0·092

(*c*) *Factors influencing the solvent action of spore filtrates.* (1) *Sterilization and bacterial action.* When a volume of spore filtrate was allowed to stand unsterilized, bacterial action quickly commenced, the solution becoming cloudy, more acid and developing

an unpleasant odour. Solutions which could not be used immediately were sterilized, experiments being made to learn whether this treatment affected the capacity of the solutions to dissolve copper.

An experiment, similar to (*b*) above, except that the solution was steam-sterilized at 10 lb./sq. in. for 30 min. before dilution, gave the following results (Table 7 and Fig. 1).

TABLE 7. *Copper dissolved by sterilized spore filtrates*

No. of spores in 50 ml. (millions)	Cu dissolved (av. of duplicates) mg.
0	0·038
100	0·050
200	0·065
300	0·078
400	0·091
500	0·105

In a second experiment spore suspensions were allowed to stand for 24 hr. and the spores removed. The filtrates were divided and used as follows: (i) used at once, (ii) immediately sterilized, (iii) allowed to stand unsterilized for 74 hr. All solutions were shaken with dried Bordeaux and the soluble copper determined (Table 8).

TABLE 8. *Copper dissolved by fresh, sterilized and contaminated spore filtrates*

	mg. Cu dissolved (av. of duplicates)		
Exp.	Fresh unsterilized solutions (50 ml.)	Sterilized solutions (50 ml.)	Solutions after standing 74 hr. (50 ml.)
A	0·035	0·034	0·032
B	0·037	0·035	0·031
C	0·053	0·053	0·037
D	0·0625	0·064	0·049
E	0·093	0·0895	0·055

Further evidence on the effect of sterilization is given below.

(2) *Temperature.* Equal quantities of a freshly obtained spore suspension were stood for 5 hr. at various temperatures, the spores filtered off, and the copper dissolved from dried Bordeaux determined (Table 9).

TABLE 9. *Effect of suspending spores at different temperatures*

Temp. ° C.	Cu dissolved by filtrate from 465 million spores (av. of duplicates) mg.
3·0	0·108
13·75	0·112
17·0	0·122
24·0	0·123
30·0	0·151

In a similar experiment performed at 13 and 23° C., determinations were also made using sterilized solutions (Table 10). The copper dissolved by a filtrate obtained immediately after cropping was 0·155 mg.

TABLE 10. *mg. copper dissolved by water in which 364 million spores had been suspended (average of duplicates)*

	Temp.	
Treatment	13° C.	23° C.
Fresh unsterilized solution	0·139	0·151
Sterilized solution	0·138	0·151
Blank	0·035	

(3) *Culture media.* Suspensions of spores grown on different nutrient agars were allowed to stand for 6 hr. at 14° C. Before using as solvents each filtrate was diluted so that 50 ml. corresponded to 400 million spores. The total solids present in the diluted filtrates were determined by evaporating measured quantities to dryness (Table 11).

TABLE 11. *Influence of nutrient medium*

Medium	Cu dissolved by filtrate from 400 million spores mg.	Total solids in filtrate from 100 million spores mg.
Bread mulch	0·173	1·92
5 % malt extract	0·144	1·68
Potato dextrose	0·115	1·05
2 % malt extract	0·103	0·93
Blank	0·023	—

(iv) *By solutions of certain pure chemical compounds*

The results obtained, using a range of 107 solutions, are given in Table 12.

TABLE 12. *Copper dissolved from dried 4:4:50 Bordeaux deposit*

Solute	Conc. of solution	Copper dissolved p.p.m.
Inorganic acids and their salts		
1. Hydrochloric acid	*N*/1000	0·67
2. Sodium chloride	*N*/1000	0·58
3. Sulphuric acid	*N*/1000	0·56
4. Sodium sulphate	*N*/1000	0·53
5. Nitric acid	*N*/1000	0·40
6. Sodium nitrate	*N*/1000	0·40

TABLE 12 (*continued*)

Solute	Conc. of solution	Copper dissolved p.p.m.
Amino acids and their salts		
7. Glycine (m.p. 240–242° (*d*))	*N*/1000	26·45; 27·17*
8. Sodium glycinate	*N*/1000	28·55
9. Alanine (m.p. 295° (*d*))	*N*/1000	24·54
10. Sodium alaninate	*N*/1000	24·81
11. *dl*-valine (m.p. 298° (*d*))	*N*/1000	27·29; 28·75*
12. Sodium *dl*-valininate	*N*/1000	30·51
13. *l*-leucine (m.p. above 300°)	*N*/1000	30·68
14. Sodium *l*-leucinate	*N*/1000	29·45
15. β-phenylalanine (m.p. 273–274° (*d*))	*N*/1000	29·46
16. Sodium β-phenylalaninate	*N*/1000	29·92
17. Glutamic acid (m.p. 200–202° (*d*))	*N*/1000	19·29; 18·71*
18. Sodium glutamate	*N*/1000	18·05
19. Aspartic acid (m.p. above 300°)	*N*/1000	17·78; 18·50*
20. Sodium aspartate	*N*/1000	18·09
21. Sodium tyrosinate (acid m.p. above 300°)	*N*/1000	19·21
22. Asparagine	*N*/1000	30·47
23. Hippuric acid (m.p. 185–187°)	*N*/1000	0·64
24. Sodium hippurate	*N*/1000	0·58
Hydroxy-acids and their salts		
25. Glycollic acid	*N*/1000	1·10
26. Sodium glycollate	*N*/1000	0·99
27. Lactic acid	*N*/1000	1·06
28. Sodium lactate	*N*/1000	0·95
29. Mandelic acid (m.p. 117–118°)	*N*/1000	1·59
30. Sodium mandelate	*N*/1000	1·52
31. Sodium benzilate (acid m.p. 150°)	*N*/1000	1·44
32. *l*-malic acid (m.p. 99–100°)	*N*/1000	15·42; 15·88*
33. Sodium *l*-malate	*N*/1000	13·32; 12·69*
34. *d*-tartaric acid (m.p. 169°)	*N*/1000	16·44
35. Sodium *d*-tartrate	*N*/1000	13·68
36. Citric acid (m.p. 152–153°)	*N*/1000	18·86
37. Sodium citrate	*N*/1000	19·36
38. Tartronic acid	*N*/1000	19·70
39. Sodium tartronate	*N*/1000	19·65
40. Gluconic acid	*N*/1000	5·72
41. Sodium gluconate	*N*/1000	6·68
Unsubstituted dicarboxylic acids and their salts		
42. Oxalic acid (m.p. 101°)	*N*/1000	1·15
43. Oxalic acid	*N*/100	4·70
44. Sodium oxalate	*N*/1000	1·07
45. Sodium oxalate	*N*/100	3·37
46. Malonic acid (m.p. 132–133°)	*N*/1000	2·95; 2·60*
47. Sodium malonate	*N*/1000	2·70
48. Succinic acid (m.p. 185°)	*N*/1000	0·63
49. Sodium succinate	*N*/1000	0·61
50. Maleic acid (m.p. 139–140°)	*N*/1000	0·77
51. Fumaric acid	*N*/1000	0·76
52. Phthalic acid (m.p. 204–206° (*d*))	*N*/1000	0·52
53. Adipic acid (m.p. 151–152°)	*N*/1000	0·49
54. Sodium sebacate (acid m.p. 132–133°)	*N*/1000	0·51
Monocarboxylic acids and their salts		
55. Acetic acid	*N*/1000	0·68
56. Sodium acetate	*N*/1000	0·59
57. Benzoic acid (m.p. 121°)	*N*/1000	0·48
Aromatic hydroxy-acids and their salts		
58. Salicylic acid (m.p. 155–156°)	*N*/1000	8·18
59. Sodium salicylate	*N*/1000	7·60
60. *m*-hydroxybenzoic acid (m.p. 200°)	*N*/1000	0·52
61. Sodium *m*-hydroxybenzoate	*N*/1000	0·50

* Represents separate shaking experiment.

TABLE 12 (*continued*)

Solute	Conc. of solution	Copper dissolved p.p.m.
62. *p*-hydroxybenzoic acid (m.p. 213°)	*N*/1000	0·54
63. Sodium *p*-hydroxybenzoate	*N*/1000	0·53
64. Sodium 2-hydroxy-3-naphthoate (acid m.p. 219–221°)	*N*/1000	3·10
65. Sodium 3-methylsalicylate (acid m.p. 162–164°)	*N*/1000	2·07
66. Sodium 5-methylsalicylate (acid m.p. 150–151°)	*N*/1000	5·91
67. Sodium 6-methyl-3-*iso*-propylsalicylate (acid m.p. 124–125°)	*N*/1000	2·36
68. Sodium 2-methyl-5-*iso*-propyl-4-hydroxybenzoate (acid m.p. 155–157°)	*N*/1000	0·49
Aminobenzoic acids		
69. Anthranilic acid (m.p. 144–145°)	*N*/1000	0·51; 0·44*
70. Sodium anthranilate	*N*/1000	0·50; 0·45*
71. *m*-aminobenzoic acid (m.p. 173°)	*N*/1000	0·44
72. Sodium *m*-aminobenzoate	*N*/1000	0·44
73. *p*-aminobenzoic acid (m.p. 186°)	*N*/1000	0·50
74. Sodium *p*-aminobenzoate	*N*/1000	0·50
Aminosulphonic acid		
75. Sulphanilic acid (m.p. above 300°)	*N*/1000	0·64
76. Sodium sulphanilate	*N*/1000	0·63
Phenols		
77. Phenol (m.p. 39–41°)	*M*/1000	0·53
78. Resorcinol (m.p. 110–111°)	*M*/1000	0·51
79. Catechol (m.p. 104°)	*M*/1000	12·52†
80. Pyrogallol (m.p. 133–134°)	*M*/1000	4·53†
Carbohydrates		
81. *l*-arabinose	*M*/100	0·45
82. Dextrose	*M*/100	0·60
83. Dextrose	*M*/10	0·55
84. Dextrose	*M*/5	0·67
85. Dextrose	*M*	4·98
86. Sucrose	*M*/100	0·62
87. Trehalose	*M*/100	0·45
Hexahydric alcohol		
88. Mannitol (m.p. 165–166°)	*M*/1000	0·49
89. Mannitol	*M*/100	0·35
α-keto acid		
90. Pyruvic acid	*N*/1000	0·95
91. Sodium pyruvate	*N*/1000	0·77
β-diketones		
92. Acetylacetone	*N*/1000	30·67
93. Sodium salt of benzoylacetone (ketone m.p. 57–58°)	*N*/1000	0·58
β-keto-ester		
94. Ethylacetoacetate	*N*/1000	1·16
95. Sodium salt of ethylacetoacetate	*N*/1000	1·60
Basic substances		
96. Ammonia	*N*/1000	0·71
97. Ammonia	*N*/100	20·50
98. Ethylenediamine	*N*/1000	20·00
99. Urea (m.p. 132°)	*M*/1000	0·64
100. Urea	*M*/100	0·63
101. Thiourea (m.p. 176–177°)	*M*/1000	< 0·05†
102. Thiourea	*M*/100	2·73†
103. Indole (m.p. 52°)	*M*/1000	0·64
104. Pyridine (b.p. 114–115°)	*M*/1000	0·73
105. Nicotine (b.p. 245–247°)	*M*/1000	0·63
106. Choline chloride	*M*/1000	0·44
107. Choline chloride	*M*/100	0·46

Glass distilled water blanks (p.p.m. Cu):

0·47, 0·44, 0·43, 0·32, 0·36, 0·37, 0·69, 0·36, 0·50, 0·72, 0·50, 0·72, 0·39, 0·59, 0·48, 0·47, 0·57, 0·55, 0·49, 0·47, 0·70, 0·85, 0·33, 0·73, 0·46, 0·58.

* Represents separate shaking experiment. † Deposit in the dish darkened during shaking.

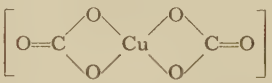
Discussion

The solubility of Bordeaux and Burgundy mixtures

Earlier work involving the determination of copper dissolved from copper fungicides was limited by accuracy of method. The modified colorimetric method used in the present work, capable of detecting copper in concentrations of 1 in 10^8, has no such limitation. Hockenyos (1931) used a diethyldithiocarbamate method for estimating soluble copper in the supernatant liquids from Bordeaux mixtures which involved the matching of aqueous solutions treated with saponin to avoid turbidity. In his experiment with 4:4:50 Bordeaux (prepared using an equal weight of lime), 1·4 p.p.m. Cu was found in the supernatant liquid after 3 days. It is not stated whether or not air was excluded. Table 1 shows that the soluble copper in 4:4:50 Bordeaux (prepared using an equal weight of slaked lime) gradually decreases on standing in absence of air, falling to 0·7 p.p.m. after 10 days.

McCallan & Wilcoxon (1936) estimated copper above 1 p.p.m. using Hockenyos's method, but for lower concentrations used Quartaroli's (1927) method. Applying this method to the determination of copper dissolved from dried Bordeaux by water, McCallan & Wilcoxon found 0·2–0·3 p.p.m., a quantity slightly less than that found in the present work (0·52 p.p.m.). The lower figure represents the copper dissolved by water allowed to stand overnight in sprayed dishes without shaking.

The liquids obtained by centrifuging freshly prepared Burgundy mixtures of increasing alkalinity (Table 2) gave figures which decreased to a minimum of about 3 p.p.m. Cu and then rose again owing to copper re-entering solution as a complex anion, which Riley (1929) suggests is of the type:



No such effect was observed using dried Burgundy deposits over the range studied (Table 3). These yielded consistently less soluble copper to water, evidence that some permanent change occurs in the nature of the precipitate on drying (see also Gröger, 1901; Reynolds, 1898; Wood & Jones, 1907; Pickering, 1909; Applebey & Lane, 1918).

Action of the leaf

In previous work on the hypothesis that the leaves of the host plant are involved in the solution of copper from dried Bordeaux (see McCallan, 1930; DeLong *et al.* 1930), only qualitative tests for copper were made. The results in Table 4 indicate that the leaves of runner beans can render copper soluble, but this interpretation ignores the fact that the sap exuded from injured leaves is capable of dissolving copper from dried Bordeaux deposit. This point was emphasized by Barker & Gimingham (1913) who suggested that the presence of minute injuries might be an explanation of Bordeaux scorch of foliage. Further, there is the possibility that the detached submerged leaves react differently to sprayed leaves on the plant. There is also evidence in these experiments that bacterial action is a factor. For these reasons, no conclusions can be drawn and work on these lines is in abeyance.

Action of the fungus spore

The results obtained, using spore filtrates, confirm the findings of McCallan & Wilcoxon (1936) that such solutions dissolve appreciably more copper from dried Bordeaux deposit than is dissolved by water, and thus support the hypothesis that the fungus spore plays an important role in the fungicidal action of Bordeaux mixture.

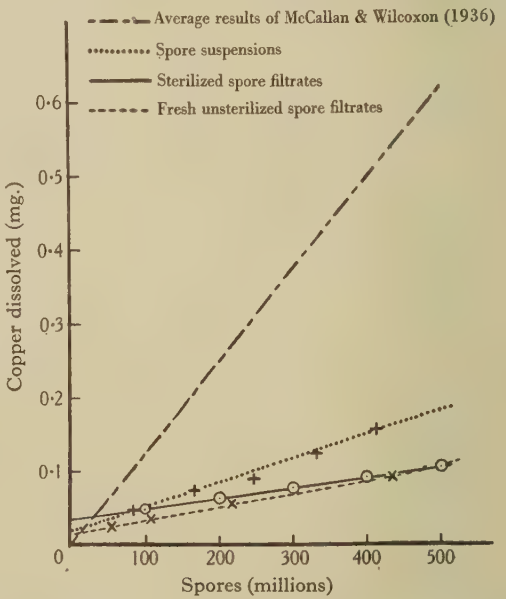


Fig. 1. Influence of *Neurospora sitophila* spores on the copper dissolved from 4:4:50 Bordeaux deposit.

The present results are compared with those of McCallan & Wilcoxon (1936) in Fig. 1. These authors worked with the spores of other species in addition to *N. sitophila* and recorded their results graphically, but in all their figures (pp. 156–157, 1936), the copper dissolved by solutions which had contained no spores, i.e. by distilled water, is represented as zero. Distilled water does dissolve small but significant amounts of copper from dried Bordeaux deposit.

During the present work, large quantities of *N. sitophila* spores were being collected for a chemical investigation on the spore filtrates, which were sterilized while awaiting attention. McCallan &

Wilcoxon (1936) did not refer to sterilization although they observed (p. 161) that 'Evaporation on the steam bath did not destroy the active principle.' Tables 8 and 10 show that sterilizing the solutions does not materially affect their capacity to dissolve copper from dried Bordeaux. Bacterial action produces a marked effect, unsterilized solutions after standing for 74 hr. dissolving less copper (Table 8), due probably to the destruction by bacteria of those substances present in the spore filtrates active in taking copper into solution. That the bulk of these 'active' substances are rapidly yielded by the spores to water was shown by the experiment in which filtrates from suspensions allowed to stand for 5 hr. dissolved no more copper (Table 10) than that from a suspension filtered immediately after cropping. This is in agreement with the more detailed findings of McCallan & Wilcoxon (1936). More copper is dissolved from dried Bordeaux by spore suspensions than by spore filtrates (Fig. 1), but the total copper dissolved by spore suspensions may exceed the quantities represented in Fig. 1 and Table 5, for the spores presumably absorb and remove from solution at least a toxic amount.

Fig. 1 shows that throughout the present work much smaller quantities of copper were dissolved by spore filtrates than were found by McCallan & Wilcoxon. The influence of factors which might be responsible for these differences was therefore studied, as the technique employed was different in the two laboratories. McCallan & Wilcoxon used 20 cm. Petri dishes sprayed with 4:4:50 Bordeaux (made using equal weights of lime and copper sulphate) and dried for at least 3 hr. Undiluted spore filtrates were then shaken continuously in the sprayed dishes for 16 hr. at their laboratory temperature (21–25° C.). The alkalinity of the Bordeaux mixtures and times of drying have been shown to have little influence on the amounts of copper dissolved, and in shaking experiments carried out at 25 and 14° C. the copper dissolved at the higher temperature was not significantly greater. The results in Tables 9 and 10 show that the temperature at which the spores are suspended in water influences the amount of copper dissolved, but it is unlikely that this effect is responsible for the differences between the present results and those of McCallan & Wilcoxon. These authors cultured *N. sitophila* on potato dextrose agar, whereas in the present work 2% malt agar was mostly employed. That the nutrient medium used has an important influence not only on the amounts of copper dissolved, but also on the quantities of soluble substances produced by the spores is shown in Table 11. Thus, spores produced on potato dextrose agar yielded slightly greater quantities of soluble solids to water than those produced on 2% malt agar. McCallan & Wilcoxon, using potato dextrose agar, found that 2·42 mg. soluble solids were extracted from 10^8 spores by water whereas only 1·05 mg. were found in the present work using this medium. This, together with the higher solubility figures obtained by McCallan & Wilcoxon, would suggest that they used a higher concentration of medium than was used by the writers, which might account for the discrepancies observed.

Solvent action of solutions of pure chemical compounds

As shown in Table 12 many of the substances studied are able to take copper into solution from dried Bordeaux deposit. With other compounds their solutions dissolve amounts of copper not greater than that dissolved by water, the maximum value for which can be taken as 0·8 p.p.m. **Hydrochloric, sulphuric** and **nitric acids** at the concentrations used (see Table 12, nos. 1, 3, 5)* occur in this group, which can be explained by the preferential decomposition by the acids of the calcium carbonate in the deposit, the resulting calcium salts being incapable of taking copper into solution. Though dried Bordeaux is readily dissolved by an excess of mineral acid, the concentrations and times of spraying were adjusted so that calcium carbonate still remained on the plate after shaking. Such conditions would presumably apply in the field where small amounts of organic substances are excreted by spores in presence of a relatively large excess of Bordeaux deposit on the leaf.

Of the organic acids studied, solutions of α-amino acids are very prominent in their capacity to dissolve copper. Most aliphatic hydroxy-acids are active and certain unsubstituted dicarboxylic acids also show this property but to a much smaller extent. In the cases of all acids which dissolve copper, approximately the same amount was dissolved by solutions of the sodium salts as by the acids themselves, again showing that this property is not associated with the acidity of the solution (cf. Sessions, 1936).

The results may be explained on the basis of the electronic theory. The nitrogen atom of the amino-group has a pair of unshared electrons which, under certain conditions, can be donated to a suitable acceptor such as the cupric ion forming a 'complex ion' different in properties to the cupric ion. Thus, e.g., cuprammonium solutions contain the deep blue complex cupri-ammine cation $[Cu(NH_3)_4]^{++}$, and in the deep blue solutions produced by dissolving the copper salts of certain amino acids in water, Riley & Gallafent (1931) gave evidence of the occurrence of a complex ion in which three anions of the acid are co-ordinated with one cupric ion, the whole complex thus having a negative charge of one:

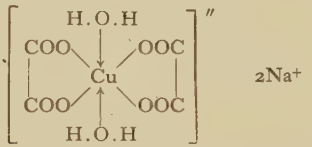
$$[Cu(NH_2.CHR.COO)_3]'.$$

In addition to nitrogen, oxygen and sulphur can co-ordinate with copper, these atoms possessing two pairs of unshared electrons.

* Throughout the subsequent paper the figures in brackets refer to the key numbers in Table 12.

Copper co-ordinated with the anions of hydroxy-acids is found in the **cupri-malates, -tartrates, -lactates** and **-salicylates,** and these complex anions, unlike the cupric ion are stable in presence of alkali (e.g. Fehling's solution).

That certain dicarboxylic acids are able to co-ordinate with copper was demonstrated by Riley (1929) who showed that of the common members of this group, oxalate and malonate ions were the most active. He suggested that in these compounds the copper was displaying a co-ordination number of six: e.g.



Such a complex is less readily formed and less stable than, e.g., the copper aminoacetate complex since in the latter the positive charge on the copper is more nearly balanced than in the bivalent dimalonato and dioxalato complexes.

Amino acids and their sodium salts

In these experiments (7–22) large amounts of copper were dissolved and the solutions after shaking were visibly blue, due to the presence of complex ion. *N*/1000 solutions of **aspartic** and **glutamic acids** and their salts (17–20) dissolved little more than half the amount of copper that was dissolved by the monocarboxylic amino acids, a result to be expected if the nitrogen atom is involved in co-ordination.

Benzoylation of glycine (**hippuric acid** (23–24)), by greatly reducing the basic function of the nitrogen atom, destroyed its capacity to dissolve copper.

Hydroxy-acids and their salts

The copper dissolved by the α-hydroxymono-carboxylic acids **glycollic, lactic** and **mandelic** and their sodium salts and by **sodium benzilate** (25–31) although more than in water, was notably less than that dissolved by the hydroxydicarboxylic acids and salts studied (32–39).

Unsubstituted dicarboxylic acids and their salts

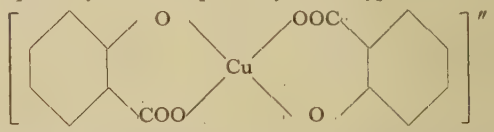
In this series co-ordination of the two carboxyl ions with Cu^{++} results in the formation of chelate rings whose size depends on the number of CH_2 groups between the two carboxyls. In accordance with Baeyer's Strain Theory extended to chelate rings, **oxalic** and **malonic acids** (42–47), involving the formation of 5- and 6-membered rings respectively, would be expected to dissolve more copper from dried Bordeaux than the other dicarboxylic acids studied. This was the case, **succinic, maleic, fumaric, phthalic** and **adipic** acids and their salts and **sodium sebacate** (48–54) being inactive at the concentrations used.

Monocarboxylic acids and their salts

Acetic and **benzoic acids** and **sodium acetate** (55–57) were ineffective in dissolving copper: the tendency of the oxygen atom to co-ordinate is weak especially when chelation is not possible.

Aromatic hydroxy-acids and their salts

The importance of chelation in the formation of a complex is well illustrated in this group, for **salicylic acid** and **sodium salicylate** (58, 59) dissolved about 8 p.p.m. Cu, whereas *m*- and *p*-**hydroxybenzoic acids** and their salts (60–63), which require the formation of 7- and 8-membered rings respectively, dissolve no more copper than water. The cuprisalicylate ion is probably of the type

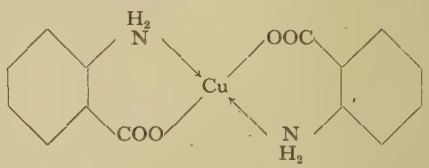


The smaller quantities of copper dissolved by **sodium 3-methyl-** and **sodium 6-methyl-3-*iso*-propyl-salicylates** (65, 67) can only be ascribed to a steric effect of the substituent groups, for the general polar influence of these groups would tend to facilitate the donation of electrons to copper.

Sodium 2-hydroxy-3-naphthoate (64), like the salicylates, was found to dissolve copper from dried Bordeaux.

Aminobenzoic acids and their salts

Anthranilic acid may be expected to co-ordinate with copper for chelation can occur:



Such a complex would carry no charge. Table 12 shows that the copper brought into solution in this experiment (69, 70) was not greater than the amount dissolved by water. During shaking, a light green insoluble compound was produced, particles of which floated on the surface of the solution. This substance could only be copper anthranilate, a compound so insoluble that it permits the estimation of copper gravimetrically (Funk & Ditt, 1933). Hence, in spite of co-ordination, the figure for water-soluble copper remains low.

Neither *m*- or *p*-**aminobenzoic acids** and their salts (71–74) nor **sulphanilic acid** and **sodium sulphanilate** (75, 76) dissolved copper, chelation being impossible.

Phenols

Phenol and **resorcinol** (77, 78) were not able to take copper into solution, but **catechol** and **pyrogallol** (79, 80), each containing *ortho*-hydroxyl groups, were both active reflecting the ease of formation of 5- and 6-membered chelate groups. It is likely that the complexes produced in the catechol and pyrogallol experiments involved the cuprous ion as both phenols are reducing agents, and evidence of the presence of cuprous oxide was obtained in the sprayed dishes after shaking.

Carbohydrates

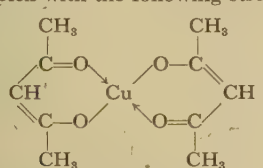
M/100 solutions of *l*-**arabinose, dextrose, sucrose** and **trehalose** (81, 82, 86, 87) failed to dissolve copper from dried Bordeaux in excess of the distilled water figure and the hexahydric alcohol **mannitol** (88, 89) was similarly inactive. Only 5 p.p.m. of copper was dissolved by a molar solution of **dextrose** (85). Bedford & Pickering (1910) showed that this substance only dissolves copper from Bordeaux containing an excess of calcium hydroxide, so that with the dried carbonated Bordeaux used in the present work, no copper would be expected to enter solution. DeLong *et al.* (1930) found that solutions of dextrose, sucrose and other sugars at 1–15 % concentrations dissolved copper from dried Bordeaux, but, as Wöber (1919) pointed out, soluble compounds between copper and sugars are only formed when a high concentration of sugar is employed. Such a concentration is unlikely to occur under field conditions and it may be concluded that sugars play no important part in the reaction between the Bordeaux deposit and the fungus spore.

α-keto acid

Pyruvic acid and **sodium pyruvate** (90, 91) dissolved less than 1 p.p.m. of copper, and since the copper salt is water-soluble, co-ordination of these substances with the copper in Bordeaux deposit cannot readily occur.

β-diketones

Acetylacetone (92) freely dissolved copper from dried Bordeaux with the probable formation of a neutral complex with the following structure:



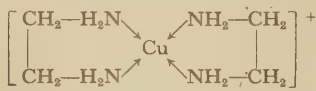
Benzoylacetone and its sodium salt should also be capable of co-ordinating with copper but this complex is insoluble in water. After shaking (93), the surface in the dish was covered with a film of this compound. The low copper figure in Table 12 does not exclude co-ordination and only represents the low water solubility of the copper salt.

β-keto-ester

The tendency of **ethylacetoacetate** to co-ordinate with copper is weak (94, 95), which is somewhat surprising in view of the high activity of acetyl acetone. The results are in agreement with the relative stability of these complexes (see Moore & Young, 1932).

Basic substances

At a concentration of *N*/1000, **ammonia** shows little evidence of co-ordination (96) although, at *N*/100, 20·5 p.p.m. of copper were dissolved (97). **Ethylenediamine** is very active at the lower concentration, 20 p.p.m. entering solution (98): it co-ordinates with the cupric ion, the complex cation possessing the high stability associated with the 5-membered chelate groups:



Except for thiourea, other organic bases were inactive towards Bordeaux at the concentrations used.

M/1000 **thiourea** dissolved less copper than distilled water (101), whereas an *M*/100 solution dissolved 2·7 p.p.m. (102). In both experiments the residue on the plate was darkened through reduction of cupric to cuprous by the thiourea. Thiourea forms complexes with the cuprous ion: e.g.

$$[Cu\ (3CS(NH_2)_2)]^+$$

$$[Cu\ (2CS(NH_2)_2).H_2O]^+,$$

so that the appearance of soluble copper must depend solely on whether or not the thiourea is present in excess. Apparently, at the lower concentration, all the thiourea was lost in the reduction, whereas at *M*/100 an excess was available for co-ordination with the cuprous ion to form a complex cation. It is thus unlikely that thiourea, if a constituent of spore excretions, plays any part in the mechanism of the fungicidal action of Bordeaux mixture. Even if present in the exudate the small amounts likely to occur would undergo complete decomposition in contact with the large excess of copper present in the deposit.

The mechanism of the toxic action of copper

It is clear from these results that the solution of copper from the dried deposit of 4:4:50 Bordeaux is independent of the acidity of the solvent, and can only occur by complex formation. Such a complex may be an anion, e.g. cuprimalate, cupriglycinate, a cation such as the *bis*-ethylenediamino-cupric ion, or a neutral complex such as is formed with acetylacetone. The degree to which a substance dissolves copper from the deposit can often be predicted from

a consideration of the structure of the compound. Generally, the presence of amino, hydroxyl and carboxyl groups faciliate co-ordination, especially in association with a structure which permits the formation of 5- and 6-membered chelate groups.

Substances which might be expected to occur in the exudate from fungus spores include amino, hydroxy and certain dicarboxylic acids and their salts and such compounds are capable of dissolving copper from dried 4:4:50 Bordeaux. Thus, since malate was found and the presence of amino acids indicated in the exudate of *N. sitophila* (McCallan & Wilcoxon, 1936), it would appear that the complex anions derived from these acids are of great importance. But although copper dissolved under the influence of spore excretions must enter solution in the co-ordinated state it does not necessarily follow that it is the complex that penetrates and proves toxic to the spore, for in solution these complexes are more or less dissociated. Thus, the cupriglycinate ion exists in equilibrium with cupric and glycinate ions as follows:

$$[Cu\,(NH_2.CH_2.COO)_3'] \rightleftarrows Cu^{++} + 3\,[NH_2.CH_2.COO'] \qquad (1)$$

This equilibrium is governed by the constant of instability K which has a value specific to the complex:

$$K = \frac{[Cu^{++}]\,[NH_2.CH_2.COO']^3}{[Cu\,(NH_2.CH_2.COO)_3']}.$$

The complex may, in this manner, serve as a bridge by which the insoluble copper fungicide enters the reaction or series of reactions leading to the production of a permeative and actively toxic derivative. The absorption of this active toxicant will remove it from the system, disturbing the equilibrium and causing these reactions to proceed if necessary until toxic concentrations are reached within the spore, a suggestion which is the general form of the hypothesis of cumulative action.

From the results of tests on a number of copper derivatives, Martin *et al.* (1942) concluded that many, including Bordeaux deposit, achieve toxicity through the agency of the cupric ion. The cupric ion, in the case of Bordeaux deposit, results from complex formation by the process outlined above, but it is not obvious how the extremely small amount of exudate yielded by spores can liberate a sufficient quantity of soluble copper to prove lethal. The results in Tables 6 and 7 indicate that the copper dissolved by the exudate from a single spore is of the order $1{\cdot}5 \times 10^{-10}$ mg. which, in a volume of, e.g., 0·01 ml. of water on the leaf, gives a concentration of only $1{\cdot}5 \times 10^{-5}$ p.p.m. Cu but these figures are misleading since they are based on experiments with spore filtrates only. In the presence of spores copper is gradually removed from solution and, if absorption of the cupric ion occurs, that part of the complex which was co-ordinated to copper [the glycinate ion in the example (1) above] would become available to dissolve a further quantity of copper from the insoluble deposit. All these reactions would proceed simultaneously in the immediate environment of the spore, but the general effect would be that 'active' substances in the spore exudate transport copper from the Bordeaux deposit to the spore wall where it becomes absorbed. The results for spore suspensions (Table 5) seem to support this hypothesis, for although in these experiments toxic quantities of copper were presumably absorbed by the spores, soluble copper remained in solution in amounts roughly proportional to the number of spores employed.

The authors express their grateful thanks to Dr H. Martin for his valuable advice throughout the investigation and to Mr R. W. Marsh and Prof. G. M. Bennett for their assistance during the preparation of this paper. They are also indebted to Dr D. D. Pratt, Director of the Chemical Research Laboratory, Teddington, for providing certain research chemicals. The work was carried out with the aid of a grant from the Agricultural Research Council.

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(*Received* 12 *April* 1943)

Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association of Applied Biologists held in the London School of Hygiene and Tropical Medicine, on Friday, 11 June 1943, at 11.15 a.m., the Chair being taken by the President, Prof. W. Brown.

Discussion on composts, their preparation and value

The following papers were read:

Composts, their preparation and value. By E. M. Crowther.
The use of composts in market-gardening. By F. A. Secrett.
The use and significance of composts in forestry. By M. C. Rayner.
The use of straw in glasshouse soils. By W. F. Bewley.

Composts, their preparation and value

By E. M. Crowther, *Rothamsted Experimental Station, Harpenden, Herts*

The word 'compost' is often limited nowadays to materials prepared by some special process, but I propose to use it in the general sense given in the Oxford English Dictionary as 'a prepared manure or mould'. In the sixteenth century Tusser used it for farmyard manure, and there is little point in narrowing its meaning.

Other speakers will describe their experience in making and using special forms of compost in market-gardening, forestry and glasshouses, and I shall therefore treat the subject in its more general aspects in relation to soil fertility. I shall try to show that composting fits particularly well into some systems of land management but not into others, that the factors involved are necessarily complex, and that nothing is to be gained by treating composting as a universal panacea. The enthusiasts who support their propaganda for some special ritual by attacking the use of fertilizers would be doing a great disservice to our present food production programme, if, in fact, notice were taken of them outside the popular press.

Almost all practical men and certainly all who are engaged in the research, advisory and educational services, have always realized that the best results, both in yield and quality of crops and in maintenance of soil fertility, can only be obtained by the proper combination of lime, fertilizers, organic manures and crop residues.

Two of the essential factors in soil fertility are an adequate supply of plant foods and the maintenance of good soil structure. It is sometimes convenient to speak of these as chemical and physical effects, but the two are intimately interlocked and they both depend on the activity of plants, animals and micro-organisms. The distinction between them is often arbitrary and their effects are difficult to analyse separately.

Relatively fresh organic matter in the coarse structural form is needed to keep soils open. Its decomposition liberates plant foods, and helps to maintain the supply of fully rotted or colloidal material. This in turn stabilizes the soil crumbs and, in conjunction with the colloidal clay, provides the soil's retentive power for calcium, magnesium, potassium and other plant nutrients.

Cultivation inevitably speeds up the losses of soil organic matter; with intensive cultivation serious soil degradation may be caused unless the losses are made good. In primitive agriculture equilibrium was maintained by alternating cropping and resting under grass or bush. In the traditional form of mixed farming in this country, where little beyond corn and meat was sold off the farm, fertility was maintained at a moderate level by rotating cereal crops with roots and leys, and, later, by purchasing imported feeding stuffs. The carbohydrates and proteins in much of the farm produce were utilized by animals; the nitrogen and other plant foods, which were recovered in the urine and faeces, served to rot down the surplus straw. It is customary to ascribe the merits of this system to the farmyard manure, and it is sometimes forgotten that the leys and root crops contributed to soil fertility in ways which could not be imitated by added organic manures. The rotation served to keep weeds, pests and diseases in check, the leys fixed atmospheric nitrogen, brought up nutrients from the subsoil and protected the soil

surface. The modern return to the methods of alternate husbandry will provide the best practical means of maintaining soil fertility, and incidentally of keeping up soil organic matter.

In well-balanced farming there is little surplus organic matter needing composting. This is the reason why farmers have hitherto taken little interest in composts and why our Research Stations have been more concerned with problems of crop rotation, farmyard manure and fertilizers.

Those who advocate the distribution of town wastes in various forms over vast distances should remember that farmers are generally disinclined to cart manure to outlying or remote fields, and that the problem of transporting surplus farmyard manure from the dairy farms of East Lancashire to the potato lands in the west of the country has not been solved. The modern tendency is to cut down unnecessary transport and handling by such methods as Mr Hosier's bail system, in which cows can be fed on or near the fields where the bulk of their food is grown. Such methods cut out the appalling wastage of nitrogen and potash on most dairy farms. The ploughing-up campaign has given many dairy farmers the opportunity of making and using farmyard manure or compost, but, for farmers who cannot keep sufficient stock, it has created the problem of utilizing surplus straw either directly or as compost.

Market-gardening, especially when it is carried out intensively with two or three crops each season, requires relatively large amounts of soil organic matter. The soil must have a good enough structure to allow work on the land at all seasons, and to withstand droughts. It must provide a large reserve of plant foods liberated steadily to keep pace with rapidly growing plants without producing a temporary and dangerous excess, as may happen from unwise use of fertilizers. The oxidation of the soil organic matter is speeded up by frequent cultivations, and the soil surface is left unprotected for many months in the year.

In the past the more important market-gardening areas developed on light soils where stable manure could be obtained from towns by road and rail. As this supply has fallen off, many market-gardeners have found it increasingly difficult to continue their old methods. A few have taken up mixed farming so that the stock can use up surplus crops and supply farmyard manure for their market-garden land. Mr R. L. Scarlett has achieved a notable success through devoting a whole season to reconditioning his fields by green manuring. He ploughs in a crop of tares after treating it with calcium cyanamide to speed up rotting and to fertilize a crop of rye, which is also ploughed in. He attaches great importance to the direct effects of the living roots in granulating the soil and to the fact that a whole season is needed to clean up weeds and pests and produce a good soil structure.

Most market-gardeners are disinclined to sacrifice a year for a rotation crop and have looked instead to ploughing-in waste vegetable products or to using materials such as peat, straw, town refuse, sewage sludge or bracken. Many of these products are difficult to incorporate with the soil and most of them differ in one or other important particular from well-rotted farmyard manure. Straw supplies a little potash, but it is so deficient in nitrogen that it immobilizes available soil nitrogen and may damage the following crop unless this deficiency is corrected. Further, it is long and tough and difficult to wet or plough in. By contrast, sewage sludge is generally wet and sticky; it supplies slowly available nitrogen and phosphate but very little potash. Straw and sewage sludge have complementary properties, and it is to be expected that by suitable composting they can be made into a useful manure. Experiments are in progress to discover the best conditions for preparation and application and to test the value of the products.

The prime purpose of composting is to rot down waste materials to a form in which they may be easily and safely incorporated with the soil. The essential requirements are:

(1) a suitable combination of materials;

(2) sufficient moisture and aeration;

(3) basic material to prevent the heap from becoming acid;

(4) protection to allow the temperature to rise sufficiently to speed up rotting, to destroy pathogenic organisms and weed seeds, and to prevent a fly nuisance.

It may also be convenient to turn the heap to save time and give a more uniform product. It is not always possible or necessary to satisfy all these requirements. Incompletely rotted compost containing free ammonia and unfermented carbohydrates can be safely used by farmers and gardeners who understand its properties. There is room for great ingenuity in fitting the technique to various conditions. Sir Albert Howard worked out an excellent but rather elaborate scheme for certain Indian conditions and has suggested a number of variants elsewhere. Others with great skill in practical composting have introduced decorative frills and mistaken these for fundamental principles. Some of the refinements are unnecessarily laborious, and, except on the small private garden scale, the labour costs are generally high. As experience grows, mechanical methods may be developed for collecting materials, making the heaps and distributing the product economically, at least for market gardens and certain special types of farming.

Much depends on the kind of waste material available. It is obviously far easier to satisfy the above conditions with a variety of vegetable waste in market gardens than on farms, and still easier in private gardens where lawn mowings, tree leaves and the cleanings from herbaceous borders can be added

to the waste from the kitchen-garden and the house. It is more difficult to make satisfactory compost from a single waste product such as straw, and it was from attempts to solve this problem that the underlying principles were first worked out by Hutchinson & Richards during the last war. They showed that straw rotted down most quickly when about 0·75 part of inorganic nitrogen were added per 100 parts of straw. This amount of nitrogen, in addition to the 0·5 part originally present, is immobilized by the fungi and bacteria which rapidly attack the cellulose and hemicelluloses. Rotting proceeds fairly rapidly till about 40% of the organic matter has gone off as carbon dioxide; subsequently decomposition proceeds much more slowly. The product has the appearance and physical properties of farmyard manure. Its nitrogen percentage on the organic matter is increased from about 1·3 to 2·3 by the rotting, i.e. the C/N ratio falls from about 40 to 20. The rotted product has inevitably a very complex constitution with most of the original lignin and other biological inert materials associated with degradation products from microbial and fungal tissues. In some properties it resembles the relatively inert humic matter of soil, except that it has only about half as much nitrogen per unit organic matter. Further decomposition and loss of carbon dioxide must take place before it reaches this still more stable form.

The bulk of the biochemical and biological work on straw composts has been concerned with the various factors in the rotting process. Much less has been done about the subsequent behaviour of the material in the soil, and especially its manurial value. It is known that most plant products, whether fresh or composted, with about 2% of nitrogen on their organic matter liberate ammonia only very slowly indeed. Those with lower proportions of nitrogen, such as untreated straw, lock up soil nitrogen. Materials with much more than 2% of nitrogen liberate some ammonia fairly rapidly. Highly nitrogenous rotted farmyard manures and composts may contain appreciable amounts of free ammonia which is readily lost on exposure to wind or rain. It is for this reason that rich manures and composts should be ploughed in as soon as they have been spread on the land.

From these considerations it is not to be expected that straw composts made with the minimal amount of nitrogen will supply much nitrogen to the following crop. A Rothamsted experiment continued over the last 9 years has shown that, starting with a given quantity of straw and sulphate of ammonia, it is far better to plough in the straw during the early winter and give the sulphate of ammonia to the following potato crop than to use the sulphate of ammonia to rot down the straw to compost. Straw used in this way increased the yield by 0·6 ton/acre and compost reduced it by 1·4 tons from the yields obtained by inorganic fertilizers alone. Both straw and compost given to sugar beet had some residual effect on the following potato crop but neither straw nor compost had any direct effect on barley grain or sugar-beet roots; they both reduced the yields of barley straw and sugar-beet tops.

At the present time it would scarcely be justifiable to purchase sulphate of ammonia to rot straw to compost on the farm scale. It would generally be better to apply the straw in autumn, preferably before a root crop, and give the extra nitrogen in the following spring. The position is quite different where straw can be used for litter in cow sheds, in fold yards, either covered or in the open, or where straw can be composted with liquid manure, which might otherwise be wasted.

In horticulture the crops normally receive large dressings of nitrogenous fertilizers. The physical effects of the bulky organic manures are so urgently needed that straw composting is to be recommended. There will, however, generally be some other vegetable waste which will facilitate the composting of straw. Under these conditions there is no need to aim at especially rich composts, provided sufficient nitrogen is given for good rotting. The composts can often be used with advantage as surface mulches, especially where the crops are to be watered.

Brief reference may be made to a few other waste materials sometimes used either alone or in composts.

Peat is probably better used as a direct addition to the soil than as an ingredient for composts, since it decomposes very slowly.

Bracken cut in July and stacked has given excellent results at Rothamsted. It supplies large amounts of nitrogen and potash. It also differs from straw in being more resistant to decay.

Sawdust presents an even more difficult problem than straw, because it is still more deficient in nitrogen and other plant foods. It has a much higher proportion of the biologically resistant organic materials and will continue to lock up nitrogen over long periods. Unless the heaps are opened up by leaves or brushwood, aeration may be difficult.

Town refuses have the disadvantage of being diluted by large proportions of inert ash, especially after wartime salvage. Most large towns burn the coarse organic matter which would be more valuable than the fine screenings.

The discussion so far has been confined to ordinary farm and garden crops, but there are other special conditions requiring different treatment. The art of making mushroom composts is well developed by specialized growers. It appears that this fungus uses the lignin and protein present in partially rotted manure. Dr Rayner's work has shown how particularly difficult soil conditions can be made more favourable for mycorrhizal fungi by adding a compost prepared from sawdust, leaves and dried blood.

So far I have said nothing about the alleged value

of hormones and vitamins in composts. Direct tests at Cambridge for vitamin B_1 in wheat with continuous manuring for over 90 years gave 20% higher values from plots with inorganic fertilizers than from those with farmyard manure. I do not think that anything can usefully be said on present evidence about those hypothetical ingredients of composts which have been said to confer perfect health and immunity from all diseases on plants, animals and man.

The use of composts in market-gardening

By F. A. Secrett, *Bell Farm, Felcott Road, Walton-on-Thames, Surrey*

Your Association is to be congratulated on arranging a discussion on composting. In view of the considerable acreage of land now coming under the plough it is desirable that this valuable process should be given full publicity. Farmers are becoming more and more conscious of the need for humus and organic manures. For many years those engaged on the scientific side of the industry have led us to believe that inorganic salts can be relied upon to supply the food necessary for plant life but, as I pointed out some years ago in a paper written for the Royal Society of Arts, tillers of the soil, owing to this teaching, have been so prodigal in the use of salts that they have been led to the brink of disaster. Reckless competition in the world food markets was largely responsible for this action, and in many parts of the world huge tracts of land have been entirely denuded of humus, a basic essential of all plant life. In some areas thousands of square miles have become barren and in other areas hundreds of farms have been literally blown away in dust clouds—all because of failure to maintain the supply of humus in the soil. Greed, thoughtlessness and ignorance, and the demand for cheap food have brought a harvest of wreckage, derelict farms and ruined farmers.

In this country, fortunately, we have not progressed so very far on the road to ruin. Lacking large tracts of virgin soil ready for exploitation, we have been compelled even in adverse conditions to make some effort to maintain the fertility of the soil. Nevertheless, our land has been robbed of humus at an alarming rate with results which are now becoming obvious.

The change from arable to grass after the last war, a change often deplored, has proved to be our salvation during this war, for in ploughing in old pastures the fertility accumulated in the turf has been brought into productive use at a most important time in our national life. This process has been accelerated by the extensive use of lime, and it should be borne in mind that the use of lime without replacement of humus is dangerous and must only be a short-term policy.

The horticultural section of agriculture has always realized the value of humus in a system of intensive cropping and quality production, but when stable manure became scarce the industry turned to organic manures such as hoof, bone meal, meat and bone, fish meal and shoddy in conjunction with sulphate of potash. As the accumulated humus in the soil resulting from previous heavy dressings of stable manure approached exhaustion, however, difficulties began to arise.

In the past most crops were healthy but with the change in the condition of the soil disease and insect pests became more prevalent year by year, and the use of dry and wet sprays became general throughout the industry; virus diseases were little recognized in my younger days, as they were not so prevalent. At one time it was considered that a shortage of lime might be responsible for some of the troubles and many growers increased the quantities applied to as high as 4–6 tons/acre. This practice further impoverished the soil and was soon discontinued. It was then generally accepted that the only satisfactory course was to resort to sprays and artificial means in the endeavour to control insect pests and disease.

A few men in horticulture still continued to use stable manure, and although they had to pay high prices for it, results justified the increased expenditure. Their farms, for all practical purposes, were free from disease and insect pests, and the produce was of high quality and in keen demand. At that period stable manure was collected in large quantities from army barracks and it was a great blow to these men when the army was mechanized, causing supplies of stable manure to become very limited indeed.

The provision of an adequate supply of humus will have to receive serious attention in the near future. Now that the need for humus is realized by the scientific side, surely it is not beyond the wit of science to devise a means of utilizing other materials as sources of humus. One important source, hitherto neglected, is the large quantity of refuse which is now either burnt or allowed to rot in the fields. Liquid waste products from animals is the most valuable manure the industry could use, yet on many farms it is carried away in the drains. It can be put to valuable use in composting.

Now that scientists and the medical profession are so keenly concerned with the safeguarding of public health it is to be hoped that their investigations and research may be extended to include consideration

of soil fertility in relation to their problems. It is possible that they may find the solution of some of these in agricultural practice.

The degree of acidity in the soil is a matter of the utmost importance in that it has a profound influence on the composition and quality of the produce grown on it, including nutritional value. There are indications that differences in the nutritional value of apparently identical produce from acid and non-acid soils are worthy of serious investigation. Acidity arises from many causes, chief of which are excessive use of inorganic salts, poor cultivations and lack of good drainage, which all lead to an improper physical condition of the soil as well.

Many years ago I came to the conclusion that the best control for insect pests was to maintain the optimum chemical, physical and microbiological condition of the soil by correct manuring and cultivations. Experience in recent years has confirmed this theory. To illustrate this point, this year salad crops have been attacked by aphis in many districts. The winter provided little frost but considerable rainfall, and consequently the land was far from the optimum condition previously described; soil acidity was accentuated. The spring weather with its droughts and wind was conducive to aphis attack, but if the soil had been in a proper condition the plants would have won the battle and grown out of their troubles. In viewing the spring crops I found that where soil was neutral and proper care had been taken the crops were free from aphis but where acid conditions prevailed infection was severe. Moreover, the aphides did not appear to migrate from acid soil to neutral soil. I have observed similar effects in the case of flea beetle on other crops, but there is no time to-day to enlarge upon this.

To maintain soil fertility has not proved easy on land which grows three or four crops yearly. The individual requirements of each crop must be considered as it is essential that they shall receive adequate and properly proportioned food supplies, in other words, a balanced diet. Nevertheless, the danger of overlooking that most important task of keeping the soil in proper physical condition is always present. No soil can be kept in good heart without humus. It aids the development of soil micro-organisms, promotes aeration, holds moisture, provides optimum conditions for root development and is conducive to a greater population of earthworms. The application of humus conforms to Nature's age-old law, from life to decay and from decay to life again, the circle completed—a law which cannot be broken without penalty.

Plant food is not limited to nitrates, phosphates and potash; plants need other substances, some in very minute quantities, and these in particular are all provided in natural humus. Science has recently discovered that boron, magnesium, manganese, copper, iodine, etc. are necessary to maintain normal plant life and that lack of them causes abnormal growth often giving the appearance of disease. It may be that there are other properties in humus-containing materials, such as stable manure, of which science has no knowledge at present.

Forty years ago stable manure was very plentiful and cheap, and sometimes market-gardeners were paid to take it away. On the other hand, they were often willing to pay 3*d.* or 4*d.* per week per horse for the manure from undertakers' horses which were all entire animals. This manure was used chiefly for making mushroom beds, as experience had proved that beds made from such manure continued in bearing 2–3 months longer than those composed of manure collected from other horses. At that period little was known of hormones, but in recent years science has shown that sex hormones may play an important part in soil fertility.

Sir Albert Howard has had great experience of composting and has carried out valuable educational work. He has been persistent in his attempts to convert a disbelieving world to acceptance of his ideas and in many quarters the value of his work is now being acknowledged.

I have composted my farm refuse with stable manure for a number of years and now that straw is so plentiful I am buying straw and composting it in the same manner. Many growers are experimenting with composting straw alone, but the chief difficulty with this method lies in wetting it properly although an atomizer used on a hose-pipe eases the process. I apply ¾ cwt. of sulphate of ammonia and about 4 cwt. hydrated lime per ton of straw. During the summer months the compost should be ready for use in 12 weeks and in this period it should be turned at least once. Composting in heaps is costly because of the labour required for turning.

Last year, in an attempt to reduce labour costs, I dressed a considerable area of poor land with 8 tons of dry straw/acre, and sprayed it by means of an irrigation plant with a solution of sulphate of ammonia. Where the irrigation plant was not available, I applied 3 cwt. sulphate of ammonia/acre on top of the straw; the fields were then disk-harrowed thoroughly to break up the straw and mix it with the soil. When this had been done sufficiently, a dressing of 10 cwt. hydrated lime was applied and the land disk-harrowed again. Decomposition took place rapidly and in about 5 weeks the material was fairly rotted down and ready for ploughing in. The best time for carrying out this work is in the early autumn when the temperature of the soil is fairly high. Crops growing on this land are looking well and fertility has been improved. I do not advocate ploughing in crude straw as this process leads to a temporary reduction of the available nitrogen. Neither am I in agreement with those who suggest complete breaking down of the humus to dust, but I do recommend a partial decomposition. Straw composted in heaps generates a high temperature and the seeds of most weeds are destroyed. When

composting is done on the soil surface the straw used must be free from weed seeds or the land will be fouled. The process naturally varies with the different materials used; I recommend breaking down vegetable waste to dust, but stable manure I would turn until it is sweet and not allow it to decompose too far.

Many of our soils, especially the alluvial sands, silts and green sands, benefit by the aeration afforded when manure is applied in a fairly coarse state. When vegetable waste is used a high temperature must be developed in order to destroy the spores of disease-causing fungi as well as weed seeds.

For a number of years I practised a form of French gardening at Whitton in Middlesex upon beds which were entirely composed of broken-down humus overlying relatively new horse manure to the depth of about 1 ft. Seven crops a year were taken from each bed in the following order: lettuce, turnips, carrots, cauliflowers, lettuce, self-bleaching celery, and radish. This was carried on year after year and disease was unknown, but no inorganics were used. I learnt much on this farm and gained valuable experience.

Present conditions have forced many growers to experiment with composting straw manure with waste materials and, it is pleasant to note, that wherever this work has been carried out efficiently, the grower has become an enthusiastic convert to the system. The high quality of crops grown on land treated with compost is so evident that even the most unobservant gardener cannot but see the beneficial effect of the treatment.

After spending 40 years of my life in the production of vegetables, fruit and flowers, I realize that even if I am spared for another 20 years I shall have little knowledge of soil fertility. At the same time, I am convinced that Nature's way of producing crops is the best way and that we have made many of our own troubles by ignoring her dictates. Now that science is giving its attention to the maintenance of soil fertility, I feel that at last we are travelling along the right road and have laid the foundations upon which our sons may build a healthy industry.

The use and significance of composts in forestry

By M. C. Rayner, *Bedford College, University of London*

My interest in composts arises from the fact that some 10 years ago I was concerned with researches as to causes of defective growth in pines and other conifers in a very poor heath soil on Bagshot Sands at Wareham Forest, Dorset. This work was published in a series of papers and details need not be recapitulated (*Forestry*, **8**, 1934; **10**, 1936; **13**, 1939; **15**, 1941).

By 1933 earlier work had led to the following conclusions, since fully confirmed:

(1) That the observed infertility was not due only to poverty in nutrients but was associated with factors directly inhibiting to plant growth, the effects reaching a maximum in early spring and declining to a minimum at the end of a fine summer.

(2) That fungal growth in this soil was directly inhibited, probably by these same factors, thus accounting for the extremely defective mycorrhizal development, although there was reason to believe that at least one mycorrhiza-former for pine was present in the experimental area.

The use of organic composts followed this diagnosis, and was directed to alter the condition of the organic substrate by making it more favourable for the growth of hymenomycete fungi; in particular those species concerned in mycorrhizal association. It was, in short, an experimental attempt at biological control.

I had had much opportunity, especially in the United States, for observing the remarkable effects produced in the mycorrhizal development and growth of conifers by experimental control of the plant residues that reached the soil in the form of prunings, slashings, etc. in mixed plantations. I had also, at that time, recently seen Lambert's work at Washington on the making of artificial composts for mushroom-growing, and had discussed with him the information then available as to the nutrient preferences of the mushroom fungus; I inferred similar preferences in other members of this group of soil fungi.

That was the background. I was not concerned with increasing the supply of available nutrients, except in respect to phosphorus deficiency which appeared then to act as a limiting factor to growth and is now known to do so. In general, I held the view that if free mycorrhiza-development could take place, nutrient difficulties would be solved automatically. This assumption proved to be correct. I may add that at that time I had not even heard of Indore composts.

With regard to the composts themselves: I was anxious to utilize waste products; I proposed to add nitrogen in the proportion of 1 % of the dry weight of the basic materials, and to standardize the composting procedure in order to obtain comparable materials for experiment.

In 1933 at Wareham Forest, randomized seed plots of three species of pine were sown with seven different composts made as follows:

Straw chaff + dried blood (C1)
Straw chaff + ammonium sulphate (C7)
Straw chaff + ammonium phosphate (C8)
Hop waste + dried blood (C5)
Sawdust: various percentage mixtures of hardwood or softwood sawdust with straw or birch leaves + dried blood in the proportion of 1% of the dry weight (C2, C4, C6).

Application: 10 lb./sq. yd. (about 2 lb. dry weight).

All these composts produced marked—often spectacular—effects on growth. Marked differences occurred in behaviour during composting and in the final products according to the sources of nitrogen used.

More recently I have used *Molinia* chaff, jute waste, bracken alone and with hops, and—since sawdust is now an embarrassing waste product, and some of this should certainly go back on the fellings—have continued experiments with various sawdust mixtures some of which are now under test in pot cultures and in the field.

The effect of compost treatment is manifest as:

(*a*) Improved growth, often spectacular in degree, without disturbance of the root/shoot ratio of growth.

(*b*) Removal of inhibition to fungal growth with marked mycorrhizal activity, followed by the appearance of sporophores of the mycorrhiza-former on all treated plots and rapid breakdown of the surface litter.

These changes appear to be self-propagating. Now, 10 years after application, with the plants still numerous on individual plots, the trees are making 18 in.–2 ft. annual growth.

All the composts contain appreciable amounts of available nutrients. When the results were published, I anticipated a challenge from soil chemists on the grounds that the growth effects were fully accounted for by the nutrient content of the composts. The challenge was duly forthcoming, backed by data from a Mitscherlich test with oats grown on several composts.

In order to test the hypothesis that the available nutrients in the composts were responsible for their effects on growth, pot experiments were set up and carried on for 2 years. Dr Robertson, then of the Macauley Institute for Soil Research, kindly supplied data as to the amount of available nutrients (N, K, P and Ca) in two composts. These experiments were done in duplicate, with and without the return of drainage from the pots throughout the whole period. Each series consisted of (1) control (untreated soil); (2) do. + standard amount of compost; (3) do. + nutrient salts equivalent to those estimated in the standard amount of compost. (For full details of the experimental procedure, see *Forestry*, **13**, 1939.) The conclusions drawn from these experiments were as follows:

(1) Drainage from this soil is deleterious to growth, the effect being apparent in the control series and masking that of the increased nutrients in the series receiving mineral nutrients. The deleterious effects disappear in the series for which soil receiving additions of compost was used. These differences, with their impact on root and shoot growth, are very clearly shown in the dry-weight records at the close of the experiment.

(2) There is healthier growth with higher dry weights of roots and shoots in the composted series as compared with the series receiving equivalent salts.

(3) Winter mortality was higher in the salt series than in the composted series, the plants in the former showing discoloration of the foliage and other signs of ill-health.

In short, these experiments showed conclusively that the increased supply of nutrients played a relatively insignificant part in the improved fertility of this soil induced by the addition of composts. They confirm previous conclusions as to the presence of soil substances deleterious to growth, and show that addition of compost puts an end to this, whereas addition of the equivalent amounts of salts is without effect.

To conclude, I hold the view that the remarkable effects on tree growth brought about by these composts on natural soils such as those at Wareham Forest and elsewhere, and also in various nursery soils, their uniformity, magnitude, and persistence over a number of growing seasons, do not depend to any extent upon the addition of nutrients, but are directly bound up with qualitative changes in the humus constituents and the biological activities related with these. Possibly also with the presence of growth-promoting substances in individual composts or produced in the soil as the result of fungal activation.

This view is dictated by mycological and not by chemical considerations. I surmise that it may not be shared by some of my audience, but I would point out that it receives independent support from other lines of enquiry in soil microbiology.

Not a great deal is known directly about the specific effects produced by particular organisms, especially fungi, in breaking down organic residues in soil. Some of the recent papers by Waksman and his colleagues are, however, significant in this connexion. There are also the interesting observations of Falck on the decomposition changes in forest residues. According to this author, the fungi commonly present in coniferous woodlands attack chiefly the celluloses and hemicelluloses, leaving the lignoproteins to accumulate, so giving rise to an acid humus, low in nitrogen, low in bases, and therefore decomposing with difficulty and leaching readily. He showed experimentally that one of the fungi

most commonly present (*Coniophora cerebella*) induced changes in the lignin compounds that rendered them unavailable to lignin-attacking forms such as *Clitocybe nebularis*, common in deciduous and mixed woodlands. In these, the fungi commonly present (as well as other organisms) attack lignin compounds and also celluloses and hemicelluloses, giving rise to humus stages more easily attacked, less acid, higher in nitrogen and bases and not leaching readily because owing to attack on the protein constituents, the salts are bound to protein residues.

These facts are very significant to anyone familiar with the rapid and drastic changes that can be induced in experimental forest plots by determining the kind of residues that reach the soil, and with the correspondingly rapid changes in the hymenomycete flora and mycorrhizal phenomena in tree-root systems that accompany these.

(The paper was illustrated by slides of photographs showing the effects of composts in seed plots, field sowings and pot cultures; the tree growth that had occurred in seed plots treated and untreated after 8 years; and the improved growth of resowings in cleared treated plots after 4 years. Photographs were also shown of plants in the experiments in which the effect of addition of composts and equivalent salts was compared.)

The use of straw in glasshouse soils

By W. F. Bewley, *Experimental and Research Station, Cheshunt, Herts*

1. *For improving worn-out soils*

The beneficial effect of mixing clean wheat or oat straw with glasshouse soils was first discovered in 1926, and since then the treatment has been applied successfully on nurseries all over the country. It arose during an investigation concerning the gradual falling off in yield which occurs when tomatoes are grown year after year in the same soil, when it was found that the yield of tomatoes could be increased by mixing a proportion of freshly cut turf with the top soil. After this, tests were made with the following materials dug into the soil at the rate of 20 tons/acre about 4 weeks before planting: (*a*) fresh grass cuttings, (*b*) the roots of turf plants, and (*c*) the ash prepared by burning the turf plants, both roots and grass. The results proved that while the incorporation of turf roots improved the physical conditions of the soil and led to improved root growth, it was the fresh grass cuttings which improved the soil most and gave the best results.

Trials on nurseries were carried out the following year and while beneficial results were invariably obtained from the incorporation of heavy dressings of grass cuttings, it was very difficult to obtain a sufficient quantity of this material during December and January. Following this, tests were made with hay, straw, and composted grass, the most promising results being obtained with straw.

In 1927 large-scale tests were made on several old nurseries, where the yield of tomatoes rarely exceeded 30 tons/acre, despite the usual manurial and fertilizer treatment. The results were outstanding in every sense, increases of 30–50 % in crop yield being obtained.

At this stage it was clear that the addition of straw to the soil was due to (*a*) an improved physical condition leading to more abundant and cleaner root production, and (*b*) increased biological activity in the soil, leading to the destruction of harmful influences, probably organic nitrogenous substances, which had accumulated in the worn-out soils. Scientific proof of this latter assumption, however, has not yet been obtained.

It soon became evident that haphazard mixing by the simple process of digging long straw into the soil did not yield the best results, because when laid in a horizontal position the straw holds water and leads to the existence of over-wet areas in which the roots decay.

Further investigation proved that best results are obtainable by placing the straw in the soil with the haulms vertical in almost vertical walls, and this can be done easily by the process of bastard trenching. The straw walls should be 2 in. apart. To be most effective they should extend to a depth of 20 in. (2 spits) and 1–2 in. of straw should stand up above the surface of the soil.

Wheat or oat straw is preferred, and trussed straw is better than baled straw as the individual straws are not crushed.

When the straw is introduced by bastard trenching it is possible to apply horse manure and fertilizers at the same time, and also sterilize the soil with a chemical sterilizer.

Applied in this way, straw improves the condition of the soil atmosphere by permitting such gases as are formed by chemical and biological processes to escape from it more readily and air to diffuse into it. It opens the soil and so encourages more abundant and cleaner root action, provides suitable food material for soil bacteria and fungi and increases the humus content. It makes heavy clay soils lighter and prevents an excess of water from lying in the surface layers. The walls of straw can be used as channels down which water can be poured into the subsoil, without applying an excess of water to the surface soil.

The process should be regarded as a means of

improving and to some extent cleaning the soil, not as a means of introducing plant foods, because in most cases the usual manurial treatment must be given in addition to the straw. It has, however, proved necessary on some sick soils, to apply straw only and so make certain of destroying the injurious influences which are retarding root growth. Later, if signs of nitrogen starvation appear in the plants, this can be corrected easily and quickly by applications of fertilizer mixtures containing dried blood.

An alternative method of introducing the straw was discovered by the late Mr C. H. Shoults of Cheshunt. It is termed the 'spade insertion' method.

The soil is prepared for planting tomatoes and then the straw is inserted as a final process. A line is stretched parallel to the double rows and about 6 in. away from the plants on the outside of the double rows. A V-shaped cut is made along the line, using a spade, the cut being about 14 in. deep. Straw cut in 20 in. lengths is laid across the top of the cut 10 in. on either side. The spade is held above the straw at a point immediately above the cut and is pressed downwards. The result is a V-shaped wall of straw in the V-shaped cut. When the work is finished there are walls of straw outside each double row and tomatoes can be planted in their proper places.

The amount of straw used is 6 tons/acre for each spit. Thus when straw is inserted in walls 2 in. thick, spaced 10 in. apart to a depth of 20 in., 12 tons of straw/acre is required.

Recently, it has been possible to purchase bands of straw 2 in. thick and of any desired width from 10 to 20 in. In these bands the straws are laid parallel to one another and are woven securely together by means of string. They have been made specially for insertion in the soil and their use simplifies the work considerably.

Walls of straw inserted into cucumber beds have also proved beneficial because they keep the compost open and maintain the roots in a healthy condition for a longer period than normally.

2. *Straw walls as a means of watering the subsoil*

Since 1926 the use of straw in the manner described has increased steadily throughout the glasshouse industry with beneficial results on the nurseries concerned, but it was not until 1940, when part of the Research Station staff moved to Worthing for 10 months' work in the Sussex area, that another use for the straw walls was recognized.

In that area the soil conditions presented many interesting problems, one of which was the persistence of dry subsoils and the difficulty of correcting them even by flooding the soil heavily with water during the winter. The walls of straw provided the means for overcoming these difficulties because if a 'spreader' is placed on the end of the hose to throw the water out as a sheet, it is a simple matter to pour all the water required down into the subsoil without saturating the top spit.

Growers have been quick to adopt this method which has led to increased crops on many nurseries. It has also proved beneficial to sweet peas and runner beans in the open, where it helps to prevent 'blossom drop'.

3. *Chopped straw as a surface treatment*

On many soils the surface forms an almost impervious crust in tomato houses, preventing the uniform distribution of water and fertilizers. Chopped straw, cavings, etc. may be used to correct this, by spreading them over the soil in a layer $\frac{1}{2}$ in. thick (6 tons/acre) and then forking them into the top 6 in. of the ground before planting. In soils low in nitrogen, it is advisable to damp the straw and scatter 2–5 cwt. sulphate of ammonia/acre over it, about a week before mixing it with the soil, otherwise the crop may show signs of nitrogen starvation at an early stage. Should this occur, however, it can easily be corrected by applying sulphate of ammonia as a top dressing.

4. *Straw as a substitute for horse manure*

During time of war good organic manure is on short supply, but straw composted by the ADCO process or with the aid of a little horse manure, constitutes a very good substitute.

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